Papers and Originals

Chromosome Studies on Women Formerly Employed as Luminous-dial Painters

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Brit. med. J., 1966, **1**, 377–382

In man the study of the harmful effects in bone of internally deposited radioactive material has been made either on subjects who have been given radium compounds internally for medicinal purposes, a practice now discontinued, or on subjects who have accidentally ingested radium, and sometimes mesothorium, while working as luminous-dial painters. The use of self-luminous paints for watch dials developed in Germany and Switzerland before the first world war and followed the discovery that scintillations of light were produced by individual alpha-particles impinging on a screen of zinc sulphide. Self-luminous paints were made by mixing radium sulphate with zinc sulphide, and sometimes mesothorium was added to the mixture.

During and immediately after the first world war increasing use was made of luminous paints, and from about 1925 reports based on the experience of early American dial-painters led to the recognition of the serious consequences that could follow the accidental ingestion of these compounds. There have also been a number of reports of the hazards associated with the internal use of radium compounds for medicinal purposes. It is clear that the accumulation in the body over a short period of time of large amounts of radium or mesothorium leads to the early production of extensive bone damage with pathological fractures, to septic necrosis of the mandible and maxilla, and in many instances to the later development of bone tumours. Severe anaemia and leucopenia are also features of the acute and subacute forms of poisoning with radium or mesothorium, as originally reported by Martland, Conlon, and Knef (1925) and Martland (1929) from studies of the dial-painters who had worked in New Jersey.

In the United Kingdom luminizing has been carried on continuously since the 1914–18 war period, and reached a peak, in terms of workers employed, during the second world war. At the beginning of the 1939–45 war it was realized that the luminizing industry would be considerably expanded during wartime, and, in view of the serious sequelae known to have occurred among early American workers, the occupation of luminizing was brought under the official supervision of the Ministry of Labour. In addition to laying down regulations designed to protect the worker against excessive radiation, this supervision took the form of periodic clinical and blood examinations, together with the maintenance of a careful watch for any symptoms that might be related to radiation exposure. Positive findings were largely confined to transient blood changes (Browning, 1949), and altogether the experience of British dial-painters, both before and after the introduction of official supervision, has not produced any reports of serious damage comparable to that observed among the earlier American workers. A more concrete long-term study of British luminizers, based on the definition of a wartime population from official records and a prospective study of subsequent experience, is under way (Boyd, 1962).

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Physical Considerations

Radium (^{226}Ra) and mesothorium, an isotope (^{228}Ra) of radium, are selectively taken up in bone, and the study of their harmful effects in man and in animals has been fundamental to the evaluation of the maximum amount of these elements fixed in bone which are considered compatible with continuing good health. Of the two elements radium has received the greatest attention, principally because of its much longer radioactive half-life (1,620 years, compared with 6.7 years for mesothorium), which allows observations to be made on contaminated individuals many years after their initial exposure. According to the recommendations of the International Commission on Radiological Protection (1959), the maximum permissible body burden of radium for occupationally exposed persons is 0.1 microcurie (μCi), 99% of this radium being fixed in the skeleton. The maximum permissible level for radium is important not only in respect of radium itself but because the maximum permissible levels of all other bone-seeking radioactive elements are closely related to it, and largely if not wholly determined by it. These elements include radioactive strontium—for example, ^{90}Sr—and plutonium—for example, ^{239}Pu. ^{90}Sr is a notorious constituent of radioactive fall-out, while ^{239}Pu is an important by-product obtained from nuclear reactors and one which is used in nuclear weapons.

Both radium and mesothorium undergo complex and differing patterns of radioactive decay through successive numbers of transmutations. During the course of these, radioactive particles and gamma rays are emitted, and it is these sources of radiant energy that are harmful to biological material. The decay of radium is initiated through the emission of an alpha-particle from an atom of radium, producing an atom of radon, a chemically inert gas. About two-thirds of the radon from radium fixed in the body is excreted through the lungs, and if the body content of radium is large enough the amount of radon in the expired air is measurable, this measurement being one method for estimating the radium content of the body. Radium decays slowly with a half-life of 1,620 years, while the half-life of radon is only 3.8 days. Radon itself, and its daughter radium-A, also decay by alpha-particle emission, and it is not until the disintegration of

radium-B and radium-C that beta and gamma rays are produced. Alpha-particles travel for only very short distances, and their presence cannot be detected by any form of monitoring equipment external to the body. However, the gamma rays emitted during the later stages in the decay of the radium series are detectable by monitoring equipment placed outside the body, thus providing a second means of estimating the body content of radium.

The measurement of radium, therefore, involves a determination of the activities of radon retained in the body and exhaled in the breath. The activity of radium is numerically equal to the sum of the two radon activities. All subjects in this report who have been employed as luminizers have been measured for gamma activity in a whole-body counter in order to estimate the retained radon fraction, and some, who were measured towards the end of the series, have also undergone measurements of radon in breath. Details of equipment and methods used have been reported elsewhere by Vennart, Maycock, Godfrey, and Davies (1965). In those cases where radon in the breath was not measured it was necessary to assume a value for the fraction of radon exhaled in order to estimate radium in the body from measurements in the whole-body counter.

According to the International Commission on Radiological Protection (1959), 70% of radon produced from radium in the body is exhaled. This value is based mainly on measurements of a small group of patients from a mental hospital who had been given injections of radium 20 years earlier. The measurements were first reported by Norris, Speckman, and Gustafson (1955), and the value 70% was later confirmed by Marinelli, Miller, and Lucas (1962). Earlier, Evans (1937) had reported measurements on a number of people in which only 45% of radon was exhaled when the radium had been in the body for more than two years. According to Mays, Van Dilla, Floyd, and Arnold (1958), the percentage of radon exhaled decreases with increasing age of the radium deposit in bone. Measurements by Vennart *et al.* (1965) on the series from which the present subjects were drawn have shown that the average exhalation is 58% of the radon produced from radium in the body. In view of the uncertainty about this factor it was decided to take a rounded value of two-thirds for the exhaled radon fraction, and, since some subjects were not measured for radon in the breath, to estimate radium in each case from the measurement of retained radon. All the radium values reported here are therefore three times the activity of retained radon measured in the whole-body counter. The standard error of measuring retained radon, due to statistical errors of counting alone, is 0.0025 μCi of radon, so that the method does not permit the accurate measurement of body radium contents of less than about 0.01 μCi.

Unlike radium, mesothorium decays rapidly, its half-life being 6.7 years and its decay being accompanied by the emission of a beta-particle. Successive disintegration products, however, emit alpha-particles as well as beta-particles and gamma rays. One of these products is the gas thoron with a half-life of only 54.5 seconds. Because of its very short half-life only a small fraction of this gas escapes from the body via the expired air. The admixture of mesothorium with radium in some of the earlier-used luminizing-paint mixtures, and to an unknown extent, can complicate considerably the assessment in former luminizers of the significance of finding tissue damage in relation to the body radium content at the time the damage is noted. If many years have elapsed since exposure to the luminous paint most of the mesothorium taken into the body will have decayed. This fact, coupled with the complexity of its decay series, makes it difficult to obtain proof of the original proportions sufficient to assess the contribution of mesothorium to the biological damage produced.

For the luminizers in this report an estimate was made of the amount of mesothorium from measurements in the whole-body counter of the activity due to gamma rays of energy 2.6 MeV emitted by thorium-C in the mesothorium decay series. The standard error of a measurement, due to statistical errors of counting alone, was about 0.0025 μCi of mesothorium. No measurable amount of mesothorium was found in any of the luminizers in this report, but, owing to its relatively short radioactive half-life, this gives no guarantee that none was present in their bodies initially.

Some radium is present in the bodies of normal adults, as minute quantities of radium are ingested in food. There is some variation in the natural body content of radium owing to geographical fluctuations in the radium content of the earth's crust, but for present purposes the average content may be taken to be 0.0001 μCi, or one-thousandth of the maximum permissible amount fixed in bone. The determination of such extremely small quantities has been made by measuring the activity of radium in samples of cremation ashes (Hursh, 1957 ; Muth, Schraub, Aurand, and Hantke, 1957).

Bone Damage

The gross changes produced in bone by all forms of radiation are the same (Vaughan, 1962). Histologically there is a direct destruction of all bone cells, the osteoblasts being apparently the most sensitive. There is also damage to blood-vessels, with secondary death of bone. Abnormal bone-reabsorption occurs and there is abnormal proliferation of connective tissue. Finally, bone tumours may develop. According to Looney, Hasterlik, Brues, and Skirmont (1955), subjects with an excessive amount of radium may show radiologically visible bone lesions when the level exceeds 0.4 μCi, or four times the maximal permissible level. However, they noted a lesion of dentine, possibly due to radiation, in the teeth of a subject with a body content of 0.15 μCi, and, more recently, possible slight bone lesions have been noted at levels of less than 0.1 μCi (Hasterlik, Finkel, and Miller, 1964).

So far, the lowest body content of radium associated with a tumour has been reported by Lucas, Rowland, Miller, Holtzman, Hasterlik, and Finkel (1963). This was in a woman who died at the age of 54 with a bone sarcoma. There was unconfirmed evidence of administration of radium at 15 years of age, and her radium content was estimated to be about 0.6 μCi. Aub, Evans, Hempelman, and Martland (1952) and Looney *et al.* (1955) have reported tumours associated with about 0.8 μCi of radium. In the former case an osteogenic sarcoma developed in a woman who had an injection of radium 25 years previously, and the latter case concerned a woman aged 74 who had never been engaged in luminizing and was unaware of ever being given radium.

To date, observations on luminizers have been directed to the study of bone damage, and, except for very high levels of body radium, little evidence of damage has been noted in blood cells when examined by conventional techniques. This report, however, provides evidence that damage to lymphocytes is observable through the study of their chromosomes and in association with small residual activities of radium in the body.

Study Population

Observations have been made on 62 women whose combined luminizing experience, while relating mainly to the 1939–45 war period, extended from the pioneering days of the occupation in this country up to the middle 1950s. They form part of a larger population of former dial-painters under current long-term study and were selected to provide a range of body contents comparable to that demonstrated by the wider survey. The 62 subjects have estimated burdens ranging from a non-measurable body radium content to a content as high as 0.56 μCi and for the present chromosome analysis have been considered in three groups according to the activity of radium in

the body, non-measurable to 0.04 μCi, 0.05–0.09 μCi, and 0.10–0.56 μCi. Table I shows the numbers of subjects in each of these groups and their mean age, mean duration of employment in the luminizing industry, mean number of years since cessation of employment, and mean body radium content (weighted for the number of cells studied). The overall age pattern reflects the nature of the group—that is, a wartime population of young females followed up some 20 years later—while the negative association between average age and amount of radium they contained is consistent with previous findings of a concentration of higher body contents among the youngest entrants to the industry (Boyd, 1962). On average, members of each group had worked in the industry for five to seven years and had ceased their luminizing employment 17 to 19 years before the present investigation.

TABLE I.—*Chromosome Studies on Luminizers : Composition of Study Groups*

Study Group	Body Activity (μCi Ra)	No. of Subjects	Average Age (Years)	Average Duration Employment (Years)	Average No. of Years since Cessation of Employment	Average Radium Content (μCi Ra)	Comments
Luminizers	0·10–0·56	9	40·3	5·9	19·2	0·39	Individual activities (μCi Ra), 0·56, 0·54, 0·53, 0·37, 0·23, 0·20 (2), 0·15, 0·13
	0·05–0·09	20	42·7	6·7	16·7	0·07	Individual activities (μCi Ra), 0·09 (2), 0·08 (4), 0·07 (3), 0·06 (6), 0·05 (5)
	Not measurable to 0·04	33	45·1	4·7	17·9	0·01	Individual activities (μCi Ra), 0·03 (1), 0·02 (1), 0·01 (14), N.M. (17)
General population*		57	50·3	—	—	—	—

* Randomly chosen women aged 35–64.

Control observations for chromosome studies were obtained from a randomly drawn group of 57 women from the list of one general practice, these women being regarded as representative of the general population of females within the age limits of 35 and 64 years. This range of age covered that of the luminizers with the exception of two subjects, though women in the control group, being more evenly distributed over the age range (Table II), had a higher average age. No subjects in either group had had therapeutic radiation exposure.

TABLE II.—*Age Distribution of Luminizers and Control Population*

Age in Years:	35–	40–	45–	50–	55–	60–	65+	Total
Luminizers	24	15	14	3	2	2	2	62
Controls ..	8	8	10	8	14	9	—	57

Technical Considerations

Chromosomes were studied in cells from blood cultures, the latter being prepared following the technique of Moorhead, Nowell, Mellman, Battips, and Hungerford (1960), the final spreading of the cells being achieved by drying in air. There is general agreement that in such cultures lymphocytes are the cells that divide in normal subjects who do not have leukaemia.

For the former luminizers an attempt was made to count and analyse 50 or 100 cells from the cultures of each subject, and in every case all abnormal cells were independently checked by a second observer. The counts were done and the results checked in ignorance of the body radium contents.

The blood samples from the former luminizers were obtained in London and returned overnight to Edinburgh as plasma suspensions of leucocytes with added phytohaemagglutinin. The samples were set up in culture within 20 to 24 hours after venesection. The samples from the controls were obtained from residents in Edinburgh. In most instances the latter cultures were established within a few hours of venesection, but in some instances up to 24 hours elapsed. The possibility has been considered that the time differences in the establishment of the cultures could be a factor in the differences in results between the controls and the luminizers. However, previous and extensive experience in setting up cultures at varying times up to 24 hours after venesection has not suggested that any appreciable difference in results can be attributed to differences in the time interval between venesection and the establishment of the cultures.

Results

Radiation produces breaks in chromosomes. The resulting discontinuities may be restored with no morphological change in the chromosomes, or the broken ends of adjacent chromosomes may join in such a way as to produce chromosomes of size and morphology recognizably different from those of the normal karyotype.

Method of Scoring

All cells have been assigned to one of three main categories:

(1) A cells, being cells without any obvious structural abnormality.

(2) B cells, being cells with a chromosome or chromosomes showing a non-staining chromatid gap, or an isochromatid gap, or a chromatid break, or a chromatid interchange.

(3) C cells, being cells with any type of structural abnormality. These cells are further divided into C_u and C_s cells.

C_u cells.—Cells carrying dicentric or tricentric chromosomes, ring chromosomes, or acentric fragments are classified as cells with unstable abnormalities (C_u cells). This is because it is assumed that the nature of the abnormality is likely to lead to the death of the cell after two or three divisions due either to difficulties encountered during division in the separation of chromatics or because of the loss of genetic material in the form of acentric fragments. It is known that exposure to large doses of radiation, either to the whole body or to a substantial fraction of the body, results in the appearance of large numbers of C_u cells. These progressively decrease with the lapse of time after exposure, but it is known that they are still detectable in significant numbers 10 or more years after exposure.

C_s cells.—Cells carrying abnormal monocentric chromosomes due to such events as the reciprocal interchange of material between chromosomes or the production of pericentric inversions are said to carry stable abnormalities. As there is no apparent reason why differences should arise during division with such chromosomes in theory, those abnormalities can be perpetuated through the process of cell division. Studies on therapeutically irradiated subjects show that they persist in significantly increased numbers for 20 years and more after exposure.

The incidence of structural abnormality among cells from the luminizer and control populations is presented in Table III. In this and subsequent tables the incidence of structurally abnormal cells is expressed as a percentage of total cells in each group. The proportion of abnormal cells in the luminizer material (4.5%) was higher than that in the control data (2.8%), and this difference remained evident when proportions of C_u and C_s cells were considered separately. While formal testing suggested that differences with respect to total C cells and C_s cells were unlikely to be due to chance, the difference relating to C_u cells failed to reach a significant level.

TABLE III.—*Proportions of C_u and C_s Cells by Study Group*

Study Group	Total Cells	C_u Cells		C_s Cells		All C Cells	
		No.	%	No.	%	No.	%
Luminizers	3,425	81	2·36	72	2·10	153	4·47
Controls ..	1,710	28	1·64	20	1·17	48	2·81
Difference between groups			0·72		0·93		1·66
		$\chi^2 = 2·91$ 1 degree freedom $0·10 > P > 0·05$		$\chi^2 = 5·64$ 1 degree freedom $0·02 > P > 0·01$		$\chi^2 = 8·36$ 1 degree freedom $P < 0·01$	

Breakdown of luminizer data by activity in the body (Table IV) demonstrated the excess of structural abnormality to be most pronounced among luminizers with the most radium (0.1 μCi and over). The proportions of C_u cells (3.2%) and C_s cells (3.6%) among cultures from persons in this group were both significantly higher than comparable proportions in the control series (1.6 and 1.2% respectively). Among luminizers with less than 0.1 μCi of radium the excess of abnormal cells was less striking, and within this range none of the individual proportions of C_u and C_s cells was significantly higher than the comparable control figure. The data as a whole, however, displayed a consistent trend of increasing structural abnormality with increase in activity of radium in the body. Thus the proportions of C_s cells, 1.2% in the general population, increased to 1.6% and 1.9% among luminizers in the low and intermediate groups, and to 3.6% among dial-painters with estimated body radium contents in excess of the current maximum permissible level (0.1 μCi). A similar gradient was evident from the data relating to C_u cells, and both trends were significant on formal testing.

TABLE IV.—*Proportions of C_u and C_s Cells by Activity of Radium in the Body*

Study Group	Body Activity (μCi Ra)	Total Cells	C_u Cells		C_s Cells		All C Cells	
			No.	%	No.	%	No.	%
Luminizers	0·10–0·56	695	22	3·16	25	3·60	47	6·76
	0·05–0·09	1,117	26	2·33	21	1·88	47	4·21
	Not meas. –0·04	1,613	33	2·05	26	1·61	59	3·66
General population	—	1,710	28	1·64	20	1·17	48	2·81
			χ^2 (trend) = 5·52 1 degree of freedom $0·02 > P > 0·01$		χ^2 (trend) = 13·98 1 degree of freedom $P < 0·001$		χ^2 (trend) = 18·52 1 degree of freedom $P < 0·001$	

In the present study cell cultures were harvested after an incubation period of either 51 ± 4 or 75 ± 4 hours, inclusive of 3 hours in the presence of colchicine. Separate analyses of these groups allowed examination for any effect comparable to that reported by Buckton and Pike (1964), who have shown that after heavy radiation exposure *in vivo* the number of C_u cells found decreases considerably with the increasing time in culture. While this finding related to conditions of radiation exposure producing large numbers of C_u cells, a similar effect was demonstrated by the data for luminizers shown in Table V. Within each range of radium contents the proportion of C_u cells was higher in the early than in the late cultures, percentages of 3.9, 2.8, and 2.3 among early cultures declining to 2.8, 1.9, and 1.5 respectively in the late cultures. The data were also consistent with a possible shift from C_u to C_s cells as culture time lengthened, in that the consistent decrease in C_u cells was paralleled by an equally consistent increase in C_s cells from 51-hour to 75-hour cultures. Thus C_s-cell percentages of 1.9, 1.3, and 1.5 increased to 4.6, 2.4, and 1.9 respectively. This pattern was not evident in the control data, which exhibited slight increases in both types of cell over the same culture period. In general, however, it did not appear that culture time, *per se*, was responsible for differences between population groups. The overall excess of C-type abnormalities remained evident within both culture times, being more marked for C_u cells among early cultures and for C_s cells among late cultures.

TABLE V.—*Proportions of C_u and C_s Cells by Culture Time*

Study Group	Body Activity (μCi Ra)	Total Cells	C_u Cells		C_s Cells		All C Cells	
			No.	%	No.	%	No.	%
Early Cultures (51 Hours)								
Luminizers	0·10–0·56	259	10	3·86	5	1·93	15	5·79
	0·05–0·09	532	15	2·82	7	1·32	22	4·14
	Not measurable –0·04	1,082	25	2·31	16	1·48	41	3·79
General population	—	647	9	1·39	6	0·93	15	2·32
Late Cultures (75 Hours)								
Luminizers	0·10–0·56	436	12	2·75	20	4·59	32	7·34
	0·05–0·09	585	11	1·88	14	2·39	25	4·27
	Not measurable –0·04	531	8	1·51	10	1·88	18	3·39
General population	—	1,063	19	1·79	14	1·32	33	3·10

Current practice in the general follow-up of British luminizers has included selective skeletal *x*-ray examination of persons containing more than 0.05 μCi of radium. Thus among present subjects, 26 out of 29 dial-painters in this range had been *x*-rayed at varying intervals prior to study of their chromosome patterns. None of the subjects with smaller radium contents had been subjected to such examinations. An increase in cells with structural abnormalities has been reported by Bloom and Tjio (1964) in blood cultures established from subjects within a short time of diagnostic *x*-ray exposures for examination of the upper or lower gastro-intestinal tract. It seems unlikely, however, that this effect played any major part in the present study. First, the degree of exposure among those with higher radium contents was relatively small. All *x*-ray pictures were taken under controlled conditions, and the selective skeletal study was usually restricted to straight *x*-ray films of hands, one or other forearm, and one or other lower leg. Less frequently an *x*-ray picture of the skull or pelvis was also taken. Secondly, if the diagnostic *x*-ray experience peculiar to the luminizers with the high radium contents had exerted any material effect on their chromosome pattern, one might expect this effect to be most pronounced among persons with a recent *x*-ray history. In practice these luminizers had been *x*-rayed at varying intervals prior to the present study, ranging from a few hours to more than four years, and it was possible to compare results relating to persons most recently *x*-rayed (less than two years) with those of others whose radiological examination was more remote (Table VI). While proportions of C_u and C_s cells, based on relatively small numbers of total cells, showed some variation among persons *x*-rayed less than two years, two years, and more than three years prior to chromosome study, there was no evidence of any excess of structural abnormality in the group with the most recent history, nor of any gradient related to time interval since radiological investigation.

TABLE VI.—*Proportion of C Cells by Time Since X-ray Investigation*

Time since *X*-ray (Yrs.)	Early Cultures			Late Cultures		
	Total Cells	C_u Cells (%)	C_s Cells (%)	Total Cells	C_u Cells (%)	C_s Cells (%)
Less than 2	199	3·0	1·0	346	2·6	3·8
2–	266	2·3	1·5	364	2·5	2·2
3 and over	236	3·4	0·8	311	1·6	4·2

Discussion

The results quoted in this communication were obtained between April 1962 and May 1963, and they must be considered in the light of improved knowledge of the requirements for

the investigation of radiation effects on cells from blood cultures. Were the studies to be undertaken now, they would be done somewhat differently. The major difference would be that the cells would not have been cultured for longer than about 45 to 50 hours, for, as has been shown by Buckton and Pike (1964) and is evident from the data in Table V, prolongation of the culture time leads to a decreased yield of C_u cells. Another difference would be that the cells would have been set up in culture within a short time of the blood samples being taken. We have no evidence that the practice of having an interval of up to 24 hours between venesection and the establishment of the culture leads to any material difference in the yields of cells, but the possibility of some effect cannot be excluded. Bearing these difficulties in mind, however, the data strongly indicate that a measurable activity of radium in the body, and certainly one in excess of the maximal permissible limit, may be associated with increased numbers of cells in blood cultures showing chromosome aberrations.

There is one feature which may in fact lead to an underestimate of the effect. One of the interesting features to emerge from the study of women randomly chosen from the general population is that there appears to be a real increase with age in the proportions of C_u cells in cultures, and there may well be some link between this and the rise that occurs with increasing age in women of the proportions of cells in cultures with a presumptive XO complement. There is good evidence that these cells arise *in vivo* through some feature, as yet unknown, associated with senescence (Court Brown, Buckton, Jacobs, Tough, Kuennsberg, and Knox, 1965a). Changes also occur in the proportions of C_u cells in ageing males, which again are possibly linked with a rise in the proportions of cells with an XO complement which occurs after 65 years of age. In the present study 32 of the 57 female controls were over 50 years old, whereas this was the case for only 9 of the 62 women formerly employed as luminizers. It is possible that if the age-structure of the control group had been more comparable to that of the study group the differences in the proportions of C_u cells would have been more emphasized.

It has already been noted that the cells which divide in cultures of blood under the stimulus of phytohaemagglutinin are lymphocytes. The interpretation of the present findings has to take account of knowledge that is now accumulating of the behaviour of these cells in irradiated subjects. The studies of Bender and Gooch (1961, 1962, 1963) and of Buckton, Jacobs, Court Brown, and Doll (1962), the former on survivors of the Y-12 criticality accident and the latter on males treated with *x*-rays for ankylosing spondylitis, have shown an important feature. This is that after radiation exposures, which are limited in time, C_u cells are detectable for many years. Among irradiated spondylitics they are present in unusual numbers 10 years and more after the end of treatment. By definition, as has already been mentioned, C_u cells are cells which have a low probability of surviving division, and in fact they are unlikely to survive more than two or three divisions. This, together with their presence in increased numbers many years after exposure, suggests that some lymphocytes have a mean survival of several hundred days, a more exact estimate being still to be achieved. This interpretation gains further support from the findings that the major proportion of C_u cells at any time after radiation exposure appear to be X_1 cells—that is, cells which from the nature of their contained abnormality are judged to be in their first post-irradiation division (for detailed discussion see Buckton and Pike, 1964).

If we postulate, therefore, that a proportion of the lymphocyte population under normal circumstances has a potential for survival without division for several hundred days, we must then consider the possible effects on these lymphocytes of the circumstances of radiation exposure from a measurable body content of radium. Spiers (1953) has estimated that the dose to the circulating blood from 0.1 μCi of radium in the skeleton is 0.0015 rep/week. Assuming a Q.F. (Quality Factor) of 10 for alpha-particles, this corresponds to a dose rate of 0.015 rem/week, which is about one order of magnitude lower than that of 0.1 rem/week recommended by the International Commission of Radiological Protection as the maximum permissible dose rate for continuous whole-body exposure under occupational conditions. A luminizer who contained 0.1 μCi of radium at the time of this investigation will have contained more radium at earlier times, and the dose rate to her blood will have been correspondingly greater. According to Norris *et al.* (1955), the fraction of radium retained in the body after a single injection is approximately inversely proportional to the square root of the time which has elapsed since it entered the blood-stream. The protracted and unknown pattern of intake of radium during the employment of these women in the luminizing industry precludes any precise estimate of their radium contents at very early times ; but even for an extreme case, when the radium might have been acquired over a very short period about 25 years prior to the measurement of body radioactivity, the radium content one year after the intake will have been only five times greater than that found at the time of measurement. There will also have been some irradiation of the blood by the radium before it was deposited in the skeleton. However, it is known (Annual Progress Report, M.I.T., 1964 ; Harrison, 1965) that after intravenous injection radium is rapidly removed from the blood. In the experiment reported by Harrison, in which a man was injected with radium–223, only 0.1% of the injected radium was present per litre of plasma 24 hours after injection. Any irradiation of the lymphocytes by radium in the circulating plasma of the luminizers must therefore have been small and been delivered over a number of years during their occupation as luminizers. There must also be a small component of dose received by the lymphocytes from the residual radium during the years that have elapsed since the women ceased employment in the industry.

On present evidence it is not possible to calculate the total doses received by the circulating blood of these women, but it seems probable that they were small and had been delivered at what must be regarded as very low dose rates. The key problem is how the observed increased frequencies of cells with chromosome aberrations are to be reconciled with exposure at such a low dose rate and the classical concepts regarding the influence of exposure rate on the production of chromosome aberrations.

Broadly speaking, the classical theory of chromosome breaks, based mainly on work on plant material, states that these remain open for a comparatively short period of time, varying according to the type of cell from a few minutes to about 20 hours, after which the breaks will rejoin. If two breaks are open at the same time, either on the same chromosome or on adjacent chromosomes and they are on average within 1 μ of each other, then an exchange of material may take place between the different parts of one chromosome or between two chromosomes, to produce chromosome aberrations. Therefore the probability of chromosome aberrations being produced by a given dose at a given dose rate is a function of the time the breaks remain open and their spatial contiguity. If the aberrations noted by us in the lymphocytes of subjects with increased body contents of radium are assumed largely to be due to the direct effect of the radiations produced by the radium and its daughter products on the circulating lymphocytes, it is perhaps somewhat surprising that a measurable yield of aberrations is present in the circumstances of exposure where the dose rate is very low indeed. Observations on men exposed to gamma rays from external sources in the course of their occupation reveal the presence of a significantly increased number of cells in early cultures that contain unstable aberrations (Court Brown, Buckton, and McLean, 1965b), and this is certainly the case when the cumulative dose, as measured on film badges, has reached about 20 to 30 rads. The dose rate for these men has not exceeded 5 rads per annum, and on average has been substantially below this figure. This

finding has in many ways been as surprising as those described among the luminizers. It may be that when the target cell is the lymphocyte, and there are indications that the half-life of these cells is much longer than had hitherto been imagined (Buckton *et al.*, 1962 ; Norman *et al.*, 1965 ; Court Brown *et al.*, 1965b), then further thought has to be given to the correctness of the classical concepts of the repair of chromosome breaks in regard both to the time that breaks can remain unrestituted and to the time in the cell cycle when restitution occurs.

Summary

Chromosomes were studied in cell cultures of blood from former female dial-painters containing radium ranging from non-measurable activities to 0.56 microcurie of radium–226. Incidence of structurally abnormal chromosomes was higher than that found among a random sample of females without luminizing experience. The study also demonstrated a consistent gradient of increasing structural abnormality with increase in the body content of radium.

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Effects of Intrahepatic and Extrahepatic Infection on Liver Function

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Brit. med. J., 1966, **1**, 382–387

Abnormalities in standard hepatic function tests may sometimes be valuable in directing attention to the liver and biliary tract in patients with unexplained pyrexia. Biochemical findings may be misleading in such patients, however, for jaundice can complicate a variety of infections, such as pneumonia (Garvin, 1836 ; Zimmerman and Thomas, 1950), typhoid fever (Stuart and Pullen, 1946), or scarlet fever (Fishbein, 1962) ; and in infants with severe infections of the urinary tract marked intrahepatic biliary retention has also been described (Bernstein and Brown, 1962 ; Hamilton and Sass-Kortsak, 1963). Furthermore, impaired bromsulphalein (B.S.P.) excretion by the liver has frequently been recorded in patients with extrahepatic infections (Machella, 1947 ; Bradley and Conan, 1947 ; Hicks *et al.*, 1948).

This paper describes the clinical and biochemical findings in six patients who presented with pyrexia of unknown origin and who later were found to be suffering from extrahepatic bacterial infections such as subacute bacterial endocarditis or perirenal abscess. None of these patients was jaundiced, yet there were profound biochemical disturbances of the liver-function tests, involving impairment of B.S.P. excretion, elevation of serum alkaline phosphatase and 5-nucleotidase, and abnormalities of other enzymes. The results in these patients are compared with those in four other patients in whom there was infection within the liver itself ; three of them had liver abscesses and the fourth was suffering from portal pylephlebitis. In these four patients similar abnormalities were demonstrated in the liver-function tests, but, in addition, the serum vitamin-B_{12} concentrations were markedly elevated, in contrast to the normal levels found in the patients with extrahepatic infection.

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Materials and Methods

Biochemical methods were those described by Wootton (1964).

Serum vitamin-B_{12} concentrations were assayed with the Z strain of *Euglena gracilis* (Hutner *et al.*, 1956). The normal range with this method is from 140 to 960 $\mu\mu$g./ml.

Liver biopsies were performed with a Menghini needle.

Patients with Extrahepatic Infection

In the six patients with extrahepatic infection abnormalities in the liver-function tests, recorded during the initial phase of investigation and before a diagnosis had been made, erroneously suggested that the source of the infection might lie in the liver or biliary tract. The clinical features, biochemical findings,