III. PROGNOSTIC INDICATORS AND BIOLOGICAL DOSIMETRY

A. PROGNOSTIC INDICATORS

207. In cases where persons are exposed to high doses, whether as a result of accidents or of irradiations for therapeutic reasons, it is essential to determine the prognosis as precisely as possible in order to be able to decide on the best treatment. The prognosis after near-lethal exposure is based on three types of data: dosimetric, clinical and biological.

1. Dosimetric data

208. Where doses to the body in general and to the bone marrow in particular can be determined with sufficient precision, it is possible to make a relatively accurate prognosis. This is the case with individuals irradiated for medical reasons or for those irradiated as a result of accidents where the distribution of the dose in the body and the dose rate are reasonably well known. Because all the dose-effect relationships suggested for mortality in man have very steep slopes, a very small shift towards lower or higher doses can cause a large variation in the probability of death. It is reasonable to assume that variability within a single species will be less than, or at most equal to, the variability between different species of similar body size. For different species of similar size, the LD_{50} varies by a factor of less than 2 ([U4] and Figures VII and XXII).

209. Taking estimates of $LD_{50/60}$ for all classes of individuals (healthy and sick), situated at the extremes of a probable range of $LD_{50/60}$ between 2.5 and 5.0 Gy (Figure XXI), and comparing them with an overall average $LD_{50/60}$ of about 3.75 Gy, for example, corresponds to probabilities of mortality of about 20% or 90%, assuming the same form of dose-effect relationship. Figure XXI illustrates these variations, showing, for example, that the 10% probability of death lies between approximately 0.5 and 3.5 Gy and the 90% probability, between 4 and 7 Gy. The large uncertainties preclude a formal prognosis only on an estimate of the dose to the bone marrow.

210. The intensive treatments to which exposed individuals are always subjected may completely change the prognosis. The treatments that are offered following accidental exposures are designed to combat intercurrent infections and aplasia, and they may increase the probability of survival. Those that are offered to patients suffering from neoplastic disorders often involve cytotoxic agents, and they may decrease the probability of survival. In the first case, the individuals are mostly healthy; in the second, the disease affecting the patients is an aggravating factor. In accidents, the higher the dose, the more intensive is usually the treatment; consequently, the slope of the dose-effect relationship may be less steep than the slope of the theoretical curve expressing $LD_{50/60}$ in the absence of treatment. It is possible that, after treatment, the $LD_{50/60}$ may be increased by a factor of (at least) 2 or (at most) 3 [L9, R6, T5].

211. The values of $LD_{50/60}$ are influenced by a variety of factors. The main ones are (a) sex: women appear to be slightly more resistant than men [F15]; (b) age: extrapolation from animals to man suggests that the LD_{50} at birth is lower than the LD_{50} for adults by a factor of 2; the value for adults appears to be attained at around puberty, with a subsequent decrease to minimum values in old age; (c) state of health: the LD_{50} is lower in individuals affected by other diseases, particularly if they relate to the bone marrow or if they reduce the natural immune responses; and, finally, the most important factor, (d) the protraction and/or fractionation of the dose with time.

212. In cases of accidental exposure, protraction and fractionation of the dose can have a very important effect; when irradiation is performed for medical reasons, whether it is whole-body irradiation or successive half-body irradiations, the dose is usually given over a few days to a few weeks. This may also be true with accidental internal exposure to long-lived radionuclides. If the dose is spread over a month or more, the LD_{50} may be increased to 10-20 Gy (see Table 19). The use of a model based on cellular responses and comparing single and multiple exposures used in radiotherapy would give a factor of 2, or an LD_{50} of about 7 Gy for protraction over two weeks [L9].

213. All these uncertainties make it very difficult to establish an accurate prognosis based solely on physical dosimetry. This is particularly true in the case of accidents, where, except for criticality accidents, the exposure time is very difficult to determine, giving rise to an additional error whose magnitude may reach

factors of 2-3 or more. Dosimetry is most valuable in the case of very low or very high doses because, whatever the possible error, one can at least establish whether the patient has been exposed in the non-lethal or the lethal part of the curve (broadly, doses up to 0.5-1 Gy or above 6 Gy).

214. The prognosis is related to the nature of the radiation involved. In accidents, one is generally dealing with penetrating radiation, since out of a catalogue of 98 accidents, 61 were caused by irradiators and 14 occurred as the result of criticality excursions in reactors [H20]. In whole-body medical irradiations, penetrating radiation is also usually involved, depending on which of the effects are desired. The prognosis is particularly difficult to establish in cases of criticality accidents with mixed gamma-neutron fields. There are two types of difficulty in reconstructing the dose: (a) the uncertainties in assessing the values of the neutron and gamma-ray components and (b) the choice of an RBE for the neutrons. The latter choice is particularly difficult, because the RBE varies according to the syndrome under consideration; in addition, the neutrons attenuate more rapidly with increasing depth than do the gamma-rays (see Figure XIX). Also, the simple addition of gamma doses and neutron doses multiplied by an RBE factor, may be an oversimplification and a source of additional error, as already discussed

215. Another important element in the prognosis is the spatial distribution of the dose. In accidents, irradiation is never homogeneous. Therefore, the concept of average dose in the bone marrow, while useful for establishing an order of magnitude, is insufficient for making a precise prognosis. Relatively small volumes of bone marrow that have escaped exposure or have been only slightly irradiated because of the inhomogeneity of the exposure are sufficient to repopulate sterilized haemopoietic areas through cell migration, as long as the marrow stroma has not been damaged.

2. Clinical data

216. An accident victim will be rapidly admitted to hospital following a reactor accident (after an accident with an isolated irradiation source it may be later before the symptoms and signs of radiation injury are recognized, depending on the dose and the part of the body irradiated). At an early stage, the critical period may not yet have been reached, and prodromal symptoms may be of major importance. The prodromal phase, described in section I.C.1, lasts from the first to the seventh day; it precedes a latency period from about day 7 to day 20 after doses resulting in the bone-marrow syndrome (Table 21). The principal gastrointestinal prodromal signs are anorexia, nausea, vomiting and diarrhoea. The average 50% incidence dose is lowest for anorexia (slightly below 1 Gy) and highest for diarrhoea (between 2 and 3 Gy). Table 22, which summarizes the results of Table 2, may allow a quick prognosis for a patient presenting one or more of these symptoms. Vomiting is an easily detectable prognostic indicator, provided that no psychosomatic factor is involved. Figure V, which expresses incidence of vomiting as a function of dose, allows a preliminary assessment of the dose level and therefore of the prognosis. In addition to defining the dose-effect relationship, the intensity of these phenomena may have prognostic value: vomiting and diarrhoea may be isolated or profuse, and they may or may not increase in frequency Their intensity and frequency are an indication of the severity. The other prodromal signs are indicators of neuromuscular reaction: fatigue, apathy, fever and hypotension (whether or not followed by hypotensive shock).

217. For doses around the $LD_{50/60}$, the most frequent prodromal indicators are anorexia, nausea, vomiting and fatigue. At supralethal dose levels, other indicators appear, such as diarrhoea, fever and hypotension [L4]. However, the prodromal indicators may occur without necessarily being followed by the death of the individual or by an acute irradiation syndrome. The latency period before their appearance is also a good prognostic feature. The earlier and more sustained is the prodromal indicator, the longer and more difficult is the return to normal, and the higher is usually the dose. Figure IV illustrates the times elapsing before appearance of the prodromal indicators; these range from a few hours for doses of around 1 Gy down to about 20 minutes or so for doses of about 10 Gy [B33]. The same data are set forth in Table 23 [I12], which also lists times of delay for the critical period (latency) and prognoses.

218. Fractionation and protraction of the dose influence the appearance and intensity of prodromal symptoms and signs. Fractionation over one to seven days increases the ED_{50} by a factor of 1.5-2.7, depending on the effect under consideration (see Figure XXIV) [L9]. Table 24 compares the ED_{50} values for the principal prodromal indicators after exposures over one day and over about a week [L9]. These doses are based on a retrospective study of 2,000 radiotherapy patients (whole-body irradiation) receiving doses above 0.3 Gy per day The ED_{10} is estimated to be about one quarter of the ED_{50}. The mean factor for exposures over a week is approximately 2; by extrapolation, it could go up to 3 for longer periods.

219. The appearance of erythema during the prodromal phase is a bad prognostic sign, particularly if erythema covers extensive areas, as this indicates a high dose. The prognosis is poorer if the erythema appears at an early stage, in spite of the fact that the patient may still appear to be in good health. For whole-body irradiations with energies of 0.1-0.5 MeV, erythema becomes manifest after doses of 2-3 Gy; with much higher energies, it will indicate higher doses at depth because of the build-up of dose in the surface layers.

220. The absence of any prodromal symptom soon after irradiation indicates an excellent prognosis: the average dose in the whole organism is probably less than 0.5 Gy and certainly less than 1 Gy. A few isolated, temporary symptoms of moderate intensity

suggest a dose below 2 Gy. From the first days after the accident onwards, the presence of clinical indicators and the observation of their severity allows a more accurate prognosis, and therapeutic decisions can be taken without waiting for the acute symptoms of the later critical phase.

221 Once the critical phase begins, the prognostic elements are much easier to interpret than they were in the prodromal phase. An excellent indicator is the time elapsing before the appearance of the critical phase; the shorter the latency time, the less favourable the prognosis All cases of accidents involving whole-body irradiation have shown this [H20]. During the critical phase, the appearance of new clinical indicators, an increase in their severity and persistence are bad prognostic signs. Table 25 lists the principal signs that may appear, classified in order of increasing severity, but not necessarily in chronological order of appearance [N4].

3. Biological data

222 The haematological syndrome presents the most serious problem for clinicians The gastrointestinal and neurological syndromes appear at considerably higher doses. 10-15 Gy in the digestive tract and 50 Gy or more in the central nervous system are required to trigger these syndromes in one week and in a day or two, respectively In the case of uniform whole-body exposure, the haematological syndrome occurs without fail below 10 Gy and down to a few Gy

223 The earliest haematological indicator is a reduction in the concentration of blood lymphocytes The speed at which this phenomenon begins is directly related to the mean bone marrow dose In general, once the fall has started, its rate, estimated over the first three days, is a good prognostic indicator [H20]. Figure XXVI shows the lymphocyte reduction in six subjects irradiated in the course of three accidents [I13].

224. Other signs are also useful, although later, biological indicators. The fall in granulocytes concentration in the circulating blood to very low levels is an important feature to be monitored, because granulocytopenia is responsible for intercurrent infections, which may cause death. Also important are the thrombocytes, which help prevent haemorrhages. Daily blood counts are the basis for the immediate prognosis and for decisions about transfusions of blood cells.

225 An important element in prognosis is the minimum level of the various blood cells and the date on which this minimum is reached (Figures X, A.II.b and A.V) In three accidents, with doses ranging from 3 to 12 Gy, the time taken to reach the nadir varied from about 4-7 days in the case of lymphocytes and from 10 days to about a month in the case of granulocytes and platelets [N5]. Quantitative data are set out in Table 26, together with the clinical outcome or the prognosis.

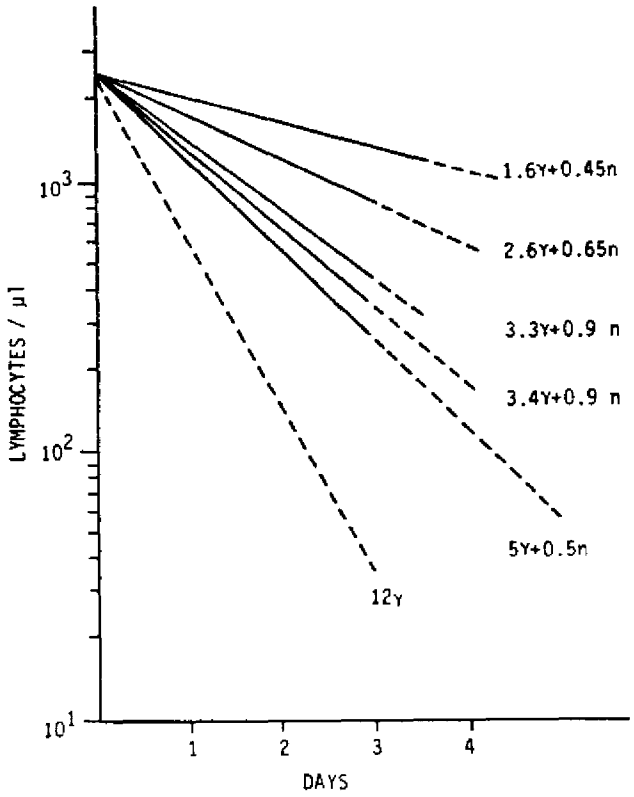

Figure XXVI. Approximate reductions in lymphocytes following accidental exposures to inhomogeneous doses. Data from six individuals exposed in three accidents: Brescia, Italy (1955): 12 γ; Mol, Belgium (1965): 5 γ + 0.5 n; Vinca, Yugoslavia (1958): 3.4 γ + 0.9 n (4.38); 3.3 γ + 0.9 n (4.18); 2.6 γ + 0.65 n (3.38); 1.6 γ + 0.45 n. Original estimates of doses (Gy) related to gamma rays and neutrons are presented separately. Values of dose in parentheses are revised equivalent low-LET marrow doses (see Table 12).
[I13]

226. After prolonged exposures it is difficult to assess the time taken for the haematological syndrome to appear, because it is difficult to fix a starting point for the irradiation period. The minimum values have the same significance for the prognosis as in the case of acute exposure; the length of time at the minimum level is more difficult to interpret for purposes of prognosis, since it seems to be related to the exposure period [H20]. Table 26 indicates minimum values of the same order of magnitude as those in Table 27 [N5].

227. During the critical phase, the duration of marrow aplasia is an important feature. A low blood count lasting for a long time is a bad sign, probably indicating not only a high but also a relatively uniform bone marrow dose and, possibly, a prolonged exposure. In the case of prolonged exposure, the depression is long-lasting and the repopulation rate is particularly slow, a mirror image of the initial slow reduction [H20]. During the phase of recovery, the reappearance of cells (whether mature or not) in the circulating blood, and their gradual increase, are good signs. Lymphocytes and platelets are generally slower in returning to normal than are granulocytes. Very often, all blood-cell types fluctuate considerably around

their normal concentrations when they return to levels within the normal range, but this phenomenon has no prognostic importance.

228. The appearance and persistence of immature cells in the circulating blood is a good sign, because it indicates a good bone marrow response The cells most frequently found belong to the granulocyte lineage: pro-myelocytes, myelocytes and meta-myelocytes. As a rule, they are present only in small numbers. It is their continuing presence over a period of days, rather than their absolute number, that is the basis for a favourable prognosis. Likewise, the number and variation over time of reticulocytes are important.

229 Other conditions such as a rare blood group, repeated transfusion problems (shock, etc.), sudden anaemia indicating haemorrhage, or leukocytosis indicating an infection, are unfavourable prognostic signs [H20].

230. A detailed examination of the bone marrow is essential for several reasons. A number of marrow punctures in widely scattered areas selected according to the conditions of the accidental irradiation (that is, the subject's position in relation to the source and the part of the body that has probably been most exposed) will give information about the uniformity of the irradiation The severity of marrow aplasia is directly related to the distribution of the dose [I14]. Marrow punctures give a much more reliable picture of the bone marrow state than does the circulating blood. However, the prognosis is not necessarily poor if the samples all show a severely depleted marrow; it requires only a few stem cells to repopulate the marrow, and direct examination with differential counting of the bone marrow cell types is an insufficient basis for a reliable medium-term prognosis It is not unusual for an apparently depopulated marrow to become repopulated to a normal level.

231. It is then necessary to perform further tests on the bone marrow cells; the cells (e.g., CFU-MIX) closely related to the stem cells should be cultured, because their existence indicates the likelihood of subsequent bone marrow restoration. There is, however, a practical problem in that such cultures take quite a long time to grow (around one week), and they require fairly elaborate techniques that cannot normally be carried out on a large scale [I14]. Furthermore, it is debatable whether they are useful in patients with extensive aplasia, in view of the relatively large marrow samples needed for the examination. Since it is exactly those individuals exposed to the highest doses who require a precise prognosis, this culture technique has its limitations.

232. Because it is easier to take blood samples than marrow samples, cultures are generally made of the circulating progenitor cells (GM-CFC). This technique has been used in cancer patients receiving partial-body or whole-body irradiation to assess injury and recovery in haemopoietic progenitor cells [T18]. There are difficulties here too, however. These circulating cells have a low concentration, the culture techniques are elaborate, and the number of GM-CFC in the blood may not adequately reflect the concentration of stem cells in the marrow. For the time being, therefore, this method remains qualitative and its true value uncertain.

233. Quantitative marrow scintigraphy can be used to evaluate the regions of the bone marrow that are still functional; this technique produces quantitative findings quite rapidly [I14, P16]. The pattern can be followed for about a week. It is possible to study, in each region of the bone marrow, the degree of iron turnover (incorporation by the erythrocytes and release by the reticulocytes) and its uptake. It is also possible to distinguish extra-medullary haemopoietic regions and to measure their relative effectiveness. However, studies such as these relate to the erythroid lineage, not to the most important granuloid lineage, and transient erythroid recovery may occur in the absence of stem-cell recovery.

234. Cytogenetic dosimetry, which may be performed in a few days, allows an estimate to be made of the mean dose in the body. Counting the number of abnormalities in circulating blood lymphocytes (mainly dicentrics, rings and fragments) and comparing this number with reference values gives an accurate estimate of the mean dose. This approach has its limitations in cases of highly inhomogeneous acute exposures during which only some of the lymphocytes are irradiated and for which the dilution factor is not known, as well as in cases of prolonged exposure. Study of the electroencephalogram is equally useful but has the same limitations with regard to prolonged exposure. All the above techniques are discussed in section III B

235. Urine analysis is useful from several standpoints (see section III.B.2) It evaluates the state of the irradiated individual's renal function, which is essential to outlasting the critical, life-threatening period It may confirm hidden haemorrhaging, by indicating haematuria, or renal malfunction associated with glycosuria or proteinuria. It is not, however, essential for determining the actual radiological damage and its consequences.

236. Biochemical analysis may throw some light on metabolic disturbances. These include disturbances that affect the regulation of the water balance, which, if extensive, can jeopardize survival. The prognosis will depend on the quality of the treatment utilized, and daily checks are indispensable. A routine check must cover (a) renal functions (urea, creatinine, calcaemia, phosphoraemia and blood ionogram); (b) liver functions (lactic dehydrogenase, transaminase, alkaline phosphatase and bilirubin); and (c) nutritional indicators (electrophoresis of peptides and proteins, and serum iron)

237. A thorough bacteriological check would make it possible, in the event that infection is discovered, to take measures that would allow a favourable prognosis, at least in the short term. The aim here would be to detect any latent infections (especially dental, otorhinolaryngeal or urinary) or opportunist infections, which are frequent in subjects with immune deficiencies. Again, the prognosis will depend on the effectiveness of the treatment. Septicaemia, fungal

infections and infections involving bacteria that are particularly pathogenic and/or resistant to antibiotics present special problems.

238. Sperm analysis is also a useful prognostic indicator. Changes attributable to irradiation are discussed in section I.D.5, and Figure XVIII shows sperm counts as a function of dose and time. The prognostic value of a sperm count is great, because the changes are very sensitive indicators at relatively low doses [I9]. A first sample must be obtained less than 40 days after the accident and a second sample, after the second month. Table 10 shows the effects of irradiation on spermatogenesis and the prognosis for fertility [L4] It should be noted that the threshold dose for permanent sterility does not rise significantly when the dose is fractionated over some days or a few weeks This is attributed to differentiation of the spermatogonia, which pass from relatively resistant early stages to type B, more sensitive, with a D_0 value in the region of 0.2 Gy [U4].

239 As is clear from the foregoing discussion, a prognosis founded on only one parameter or one class of parameters (dosimetric, clinical or biological) is bound to be very uncertain. To be valid, a prognosis must be founded on an entire range of data, and the wider the spectrum of these data and the better their coherence, the more refined will be the predictions. Table 23 summarizes the kinds of data that are useful in prognosis [I12], and Table 28 recapitulates the threshold levels of the signs and symptoms that can be detected by specialist teams and that appear after low doses [N5].

B CLINICAL AND BIOLOGICAL DOSIMETRY

240 The many ways of estimating dose can be divided into two main kinds of investigation: (a) clinical dosimetry, which compresses the observation discussed in sections I C and I.D (the relative value of these observations is discussed in section III A) and (b) biological dosimetry, which comprises all the laboratory examinations that might allow an evaluation of the dose received by the individual, its distribution in the body, the time span of dose delivery and the quality of radiation involved. Biological dosimetry relies on haematological, biochemical, cytogenetic and neurophysiological examinations [E10, I13, J11, J12, K19, N14], which have different degrees of dosimetric value. Some are only qualitative (biochemical examinations, for example), others have considerable prognostic value (cytogenetic and neurophysiological examinations, for example), the majority are difficult to interpret in cases of protracted or fractionated exposures.

1. Dosimetry based on haematological data

241. Quantitative morphological haematology (cell count, differential count, platelet count etc) is discussed in sections I.C.4. and III.A. Irradiation causes changes in the circulating blood components (cells and plasma) and in the haemopoietic tissues. and the examinations have to be more rigorous than routine examinations

242. The morphology of the cells can be changed by irradiation. Frequently, the number of binucleate lymphocytes is higher than normal [H20, J13, J14, R17]; however, their appearance is generally delayed, so this measure is of little interest for immediate diagnostic purposes. Other abnormal features have been observed in the peripheral blood lymphocytes of persons irradiated with high doses, including (a) nuclear changes, (b) nuclear pycnosis; and (c) micronuclei, which are the result of chromosomal abnormalities but can be detected more easily and more quickly than karyotype abnormalities. These changes can be observed at relatively low doses, typically 0.25 Gy in vivo and 0.02 Gy in vitro [I15].

243. The number of peripheral lymphocytes displaying a defective nuclear structure has been shown to be related to dose for doses above a few Gy, administered in vitro and in vivo [W14] This phenomenon has been studied in rats and in humans, but it cannot be readily used for dosimetric purposes because the damaged cells are trapped by the reticulo-endothelial system and rapidly disappear from the bloodstream; blood samples must therefore be taken soon after irradiation and incubated in a culture medium for several hours

244. The incidence of nuclear pycnosis in lymphocytes irradiated in vitro is also related to dose. In animals (rats, rabbits) there is a linear relationship up to about 1 Gy, with a low threshold at about 0.05 Gy [I15] However, this method is unreliable because pycnotic lymphocytes vary so widely among non-irradiated subjects Moreover, it has been shown that in animals, pycnosis varies with the size of the cell and the nucleus/cytoplasm ratio [R18].

245 At doses between 1 and 8 Gy [I15], irradiation reduces the uptake of tritiated thymidine in vitro by the lymphocytes following treatment with phytohaemaglutinin. This explains the reduction in mitoses and cellular transformations during irradiation. Although it should be regarded as only semi-quantitative, this method is sometimes used in cases of accidental irradiation [W15].

246. Irradiation also affects the electrophoretic mobility of lymphocytes and the distribution of cellular volumes [S25] This was noted in rabbits after doses of 2 and 4 Gy, where there was an increase in the number of large cells in the second week after irradiation. At the same time, in the categories of cells characterized by their degree of mobility, the category showing the first changes manifested the phenomenon only briefly, starting about five minutes after exposure and lasting for about 30 minutes. The explanations offered for this vary: an increase in cellular metabolism that produces functional changes in the lymphocyte or, alternatively, differences in the cell populations at the outset, with the most mobile groups able to undergo certain alterations outside the circulation and then to reappear with a different volume [I15].

247. Various immunological changes have been measured after regional irradiation [W17], in surface markers, mitogen and antigen responses and cytotoxic functions (see section I.C.4). Some of these changes persist for up to 10 years, but there have been no detailed studies of their potential use as biological indicators of radiation dose.

248. Leucocytes other than lymphocytes also show malformations after irradiation. There are few quantitative data, and such data as there are, are of little use in establishing a diagnosis or prognosis These changes only confirm exposure; it is not so far possible to correlate them with dose or to know their relative importance. The most common changes are (a) giant polynuclear cells (hypersegmented polyploid granulocytes); (b) cells with small alterations in their nuclear structure, such as small chromatin adnexa; (c) the presence of immature granulocytes; and (d) mitotic abnormalities in erythroblasts and granulocytes [B16, F5, I15, I16]. These abnormalities occur only infrequently. The reduction in the number of monocytes may be related to the inhomogeneity of the dose, in the sense that when severe monocytopenia appears rapidly, it is a sign that a very large proportion of the bone marrow has been irradiated [I15] Conversely, if a significant part of the haemopoietic marrow has escaped irradiation, there may be only a temporary reduction in the number of monocytes, or even monocytosis with an increase in the number of immature cells [I16].

249. Erythroblasts can be found in the blood stream after irradiation, always in very low proportions [B16, I15] Reticulocytes are useful indicators in prognosis [H20], a dramatic fall in reticulocyte count is often a sign of early fatality.

250. Serum glycoproteins increase in the presence of infection, inflammation or neoplastic and idiopathic disorders. The effect of irradiation on the concentration and distribution of protein-bound carbohydrates in the serum of mice and dogs has been studied [I15]. A considerable increase has been reported in animals exposed to lethal doses, while no notable change has been reported in animals receiving lower doses. It is not possible to treat all glycoproteins as one entity to be used as a dosimetric indicator. However, following the separation of various elements, changes in concentration have been noted for transferrin, haptoglobulin, the β_2 glycoproteins and the α_2 macroglobulins [E8]. A common feature of all these proteins is their richness in bonded carbohydrates.

251. Because the bone marrow function is extremely important for prognosis, all tests of its proliferative ability may a prion be regarded as useful dosimetric indicators. The analysis of bone marrow cannot in any case be a substitute for studies of the peripheral blood, which are currently the most reliable biological dosimeter.

252. The mitotic index in bone-marrow cells is one of the most accurate biological indicators, and also has a certain prognostic value. Changes in the mitotic index are related to the dose, but doses of 1 Gy or lower produce little change [K12]. After a few Gy in man, there is an initial drop in the mitotic index, recovery at about day 8 and a further fall before it returns to near normal by day 24 [F9]. In the Y-12 accident, the marrow of individuals who had received estimated doses of 2.4-3.7 Gy had practically no mitotic cells. For higher doses, the cell count dropped slightly from day 3 after exposure. The most commonly observed morphological change was the presence of giant neutrophil precursors from day 2 to day 16.

253. Some authors have proposed a test based on the marrow's capacity to respond to stimulation: injection of ethiocholanolon causes granulocytes stored in the bone marrow to migrate into the bloodstream [G18, I15, V15]. The ethiocholanolon is a testosterone metabolite, δ-4-androstane-3, 17-dionin. It is an androsterone isomer, with the configuration 5β-H (A:B cis); androsterone has the configuration 5α-H (A:B trans). The ethiocholanolon test was first used in patients suffering from malignant blood disorders; given the relationship between the responses to this test and the quality of the peripheral granulocytic cell pool, it was considered to be a good guide to the therapy of these blood diseases [V15]. Furthermore, ethiocholanolon affects neither the mononuclear nor the thrombocytic cell lines. The granulocyte outflow starts quickly and lasts for about 16 hours after the injection. Because of its low toxicity and the good reproducibility of the response, the method using ethiocholanolon is superior to methods using other leucocyte-mobilizing agents. A positive response indicates active medullary production of granulocytes.

254. Many of these methods have now been superseded by cell culture techniques for bone marrow. These techniques are at present fairly reproducible, mixed-cell colonies originating from cells closely related to the stem cells and granulocyte/macrophage colonies arising from granulocytic precursor cells may appear in cultures even using marrow punctures that have indicated, morphologically, a lack of haemopoiesis. Quantitative medullary scintigraphy, in conjunction with the iron-59 test, gives a picture that can be used to assess the impairment of the marrow.

255. Because the mature cells are resistant and have a long lifetime (approximately four months), it is difficult to use erythrocytes directly as early biological indicators of radiation damage, although the erythroid precursor cells are radiosensitive. Iron is incorporated only into the precursor cells, and the iron-59 test, in conjunction with the other tests of medullary function, allows the erythroid cell populations to be evaluated.

256. A greater denaturation of haemoglobin in erythrocytes by phenylhydrazine was reported in occupationally exposed persons, compared with the normal population [G22]. In patients with bronchial carcinoma given fractionated doses (1 2-1.5 Gy per day), an increased denaturation was observed when the cumulative dose reached 7-9 Gy [G22]. However, no increase was noted when erythrocytes from normal individuals were given doses between 1 and 8 Gy in vitro [G23].

2. Dosimetry based on biochemical data

257. Any changes in the blood biochemical parameters may be regarded as interesting signs. Glycaemia cannot be taken as a biological indicator because of its high degree of stability in the body. It is not unusual to observe hyperglycaemia from day 1, followed by pronounced hypoglycaemia (0.5 g/l) on about day 3 and a return to normal that takes about a week, after some fluctuation around the normal level [J13, J14, J15]. In the same way, fluctuations in plasma electrolytes and plasma proteins increase as the dose rises. However, the data are not accurate enough for these indicators to be used quantitatively [J15]. The features often noted are disturbances such as hypochloraemia, hyponatraemia or hypokalaemia during the first week [J13]. Electrophoretic analysis of the protein fractions shows the largest reduction (greatest at about two weeks) in the albumins. A dose-dependent appearance of a humoral factor in blood serum, which inhibits incorporation of ^{125}IUdR into cells in culture, was reported in mice [F7]. The technique has not yet been developed for man, partly because the thymidine concentration is only one-tenth that in mouse serum and partly because of other technical difficulties [F8, S29].

258 Hyperamylasemia can be produced by irradiation [B51, C38, K20, T30] The pancreas is not very sensitive (doses up to 2 Gy have no effect), but amylase increases are detected if the salivary glands have received more than 0.6 Gy [W18]. The increases are maximal on day 1 after radiation, returning to normal by day 3, but a clear dose-dependence has not yet been established

259. The variations in the chemical composition of the urine are of more significance than those of the blood. The urinary electrolytes may reveal changes in potassium excretion (extra-physiological fluctuations) and in the excretion of sodium and chlorine, which declines during the first few days after exposure [J13] The 17-ketosteroids increase substantially during the first few days, before returning to normal by the end of the first week [J13]

260. After radiation exposure, there is a considerable enzymatic breakdown of nucleic acids and proteins, especially in lymphatic tissues [A29, H42, S38] As a consequence, the urinary excretion of nucleosides and amino acids, as well as their metabolites, increases. A dose-dependent increase of deoxycytidine from normal low levels [I15] was observed in the urine of rats during the first day after a whole-body irradiation with 0.5-2.5 Gy [G30]. An enhanced excretion was also found in man after radiotherapy [B55, S39]. Similar effects were reported for the excretion of thymine in rats [Z5]; however, this was not seen in man [B55] Thymine is metabolized to β-aminoisobutyric acid (BAIBA). The excretion of this substance is considerably increased in mice, rats and man after irradiation [S39] After accidental human irradiation, an increase from 100-200 μmoles per litre of urine to 250-650 μmoles per litre was observed [G19, J14].

261. There is a general increase in the levels of amino acid in the urine of animals and humans during the first day after irradiation [S39]. The relative enhancement depends on the absolute excreted amount and on the metabolism of the specific amino acids. Because these factors are very complex and different for each amino acid (a decrease in urinary excretion can occur with some) no general rule is observed [S39, J14]. Accordingly, the excretion of amino acids is not usually an appropriate indicator.

262. There are, however, some amino acids or their metabolites that show a dose-dependent change in urinary excretion after irradiation. One of these is taurine, which is the metabolic end-product of cysteine. Its excretion increases 1-2 days after irradiation in the urine of rats and mice [K22, S26, S38]. Excretion increases with radiation dose in the range 0.75-2.5 Gy. In man, an enhanced urinary level of taurine was also observed after accidental irradiation [A29, J14]. It has been suggested that the increased excretion of taurine after irradiation may be related to intracellular taurine elimination due to changes in cell permeability [S26] and to the breakdown of lymphatic tissues [D12]. However, metabolic studies in mice show that the biosynthesis of taurine is also altered [H42, S40].

263. Some days after irradiation the urinary excretion of taurine decreases below normal values [L30] This effect is due to metabolic changes of vitamin B_6-dependent decarboxylases and other enzymes which are decreased, as in the condition of vitamin B_6 deficiency [S38] As a consequence of such metabolic alterations, the urinary excretion of kynurenic acid and xanthurenic acid (metabolites of the amino acid tryptophan) increases after irradiation of mice and rats [A29, H42, L30, S38, S39]. This effect was also observed in man [L29] These changes occur in a dose range of 4-8 Gy, which, in mice and rats, causes severe radiation sickness prior to death [S39]. From these studies it can be concluded that biochemical indicators may be useful for certain dose ranges Thus, the breakdown products of nucleic acids and taurine may be useful indicators in a lower dose range (0.5-3 Gy) and metabolites like kynurenic and xanthurenic acid, in a higher dose range (4-8 Gy).

264. Creatinine could serve as a measure of radiological damage to the irradiated muscles that are no longer able to metabolize it in the normal way [G20]. The level of creatinuria has never been correlated with dose, but it may confirm the uniformity of irradiation. In accidents where a relatively large portion of the body has not been irradiated, such as the Lockport accident in 1960, the level of creatinuria (creatine/creatinine ratio) scarcely increased. In accidents involving whole-body irradiation, such as occurred at Oak Ridge (Y-12) in 1958 and at Mol in 1965, the level was significant, with three conspicuous peaks in one instance (day 2, end of first week and end of second week) [J14].

265. In recent years a number of new biochemical indicators of severe radiation damage have been proposed. For example, a method has been suggested for the quantitative evaluation of damage to the

membranes of erythrocytes in the peripheral blood [M50, M51, M52]. Inhibition of the incorporation of labelled precursors of the DNA in bone-marrow cells has been used in a method worked out by Porschen et al. [P31]; the authors used their method to monitor irradiation even in small doses (some tenths of Gy). There are also data on determining the total content of desoxyribonucleotides in the blood and urine of patients irradiated for therapeutic purposes [S46, T31]. However, due to the paucity of such studies, it is difficult to evaluate the value of these indicators.

3. Dosimetry based on cytogenetic data

266. The analysis of chromosome aberrations in the circulating lymphocytes is widely used to assess the dose. Even in cases of partial-body exposure, the chromosome changes are excellent indicators of the absorbed dose. The evidence to justify the technique is well founded and covers various irradiated populations (in nuclear medicine, radiotherapy and accidents) and very wide ranges of dose [B42, K13, L15, L16]. The technique provides a reliable indication of the acute dose, since lymphocytes are widely dispersed in the various tissues and organs, have a reproducible radiosensitivity and a long life, and circulate rapidly in the body [D13].

267. Many types of radiation-induced chromosomal aberrations may appear in irradiated lymphocytes, but the dicentric aberration is currently taken as providing the most valuable information on dose This is because the dicentric aberration is almost unique to ionizing radiation and occurs rarely in persons exposed only to normal background radiation. Centric rings occur only 5-10% as frequently as dicentrics in control or irradiated lymphocytes and are thus too infrequent to be used as a sole measure of dose. Some researchers combine the centric and dicentric yields. Acentric fragments, by contrast, have a higher background frequency, which probably reflects their induction by a large number of chemical mutagens. The confounding effect of many environmental non-radiological insults reduces the acentric's value as a measure of dose, although elevated acentric yields may qualitatively support dose estimates derived from the dicentric incidence.

268 Human T-lymphocytes have a long lifetime; a small proportion of them survives for decades. The rate of replacement is quite slow, so that in the few weeks after exposure the dicentric yield remains fairly constant. After a partial or inhomogeneous acute exposure, the lymphocytes that were in the irradiated volume of the body in both the vascular and extravascular pools are rapidly mixed with unirradiated cells. An equilibrium is reached by about 20 hours [T25], and thereafter the dicentric yield in cells from a sample of peripheral blood will provide an estimate of the average whole-body dose.

269. The dose-response for dicentric aberrations in the irradiated lymphocytes of normal individuals is little affected by factors such as the donor's age or sex. The dose-response obtained for irradiation in vivo does not differ significantly from that for irradiation in vitro [C46], so that the aberration yield observed in cells taken from an irradiated subject may be interpreted by reference to the appropriate calibration curve in vitro. In vitro curves have been established for a large range of radiation qualities, including all those likely to be encountered in accidents [L25]. Within any one laboratory, the calibration curves for dicentrics have proved to be very reproducible, provided that the cells are examined at their first post-irradiation mitosis. This is now reliably achieved by including bromodeoxyuridine in the culture medium and staining the chromosomes by fluorescence plus Giemsa [S37].

270. For low-LET radiations, the yield of aberrations, Y, conforms well to the quadratic relationship $Y = c + \alpha D + \beta D^2$ where c is the background incidence (about one dicentric in 10^3 cells), D is the dose, and α and β are fitted coefficients. A dicentric aberration requires the interaction of two breaks, each induced in separate G_0 or G_1 chromosomes. An explanation of the quadratic relationship may be that when both breaks are produced by the passage of a single ionizing track, the yield is represented by the linear term αD. The βD^2 term thus represents those dicentrics that are produced when the two breaks are caused by separate ionizing tracks. The latter term becomes more important when the dose increases

271. In general, high-LET radiation, such as fission spectrum neutrons and alpha particles, give a linear dose response relationship, $Y = c + \alpha D$ (Figure XXVII). For these types of radiations the ionizing events are so densely distributed along the track that there is a high probability that one track will deposit energy in both chromosomes. RBE values at low doses, calculated as the ratios of the alpha coefficients of two radiations of different quality, may represent the relative hazards of the two radiations at low routine occupational levels [I21] At higher doses, such as are likely to cause overt symptoms of sickness, the values of RBE decrease markedly [L36]. With neutrons, as their energy increases the average LET decreases and the linear model requires a second (quadratic) term, e.g., with 7.6 MeV and 14 7 MeV neutrons RBE values for specific energies of neutrons have been proposed [B44, P18].

272. Another feature of the dose-response curves for high- and low-LET radiation is the relative importance of the dose rate. For high-LET radiation with a linear dose response, this is unimportant. For low-LET radiation, the equation $Y = c + \alpha D + \beta G(x) D^2$ can be used, where the number of initial chromosome breaks falls exponentially with time according to the factor $G(x)$. In practice, the dose-squared term reduces until the response can be considered to be linear for x- or gamma-radiation doses of a few Gy, if delivered at a more or less uniform rate over 24 or more hours.

273. An important problem in assessing the dose to an appropriate degree of precision is the number of metaphase cells that have to be examined. As a rule, evaluation of 100-500 metaphases is sufficient to estimate a dose at irradiation levels of medical

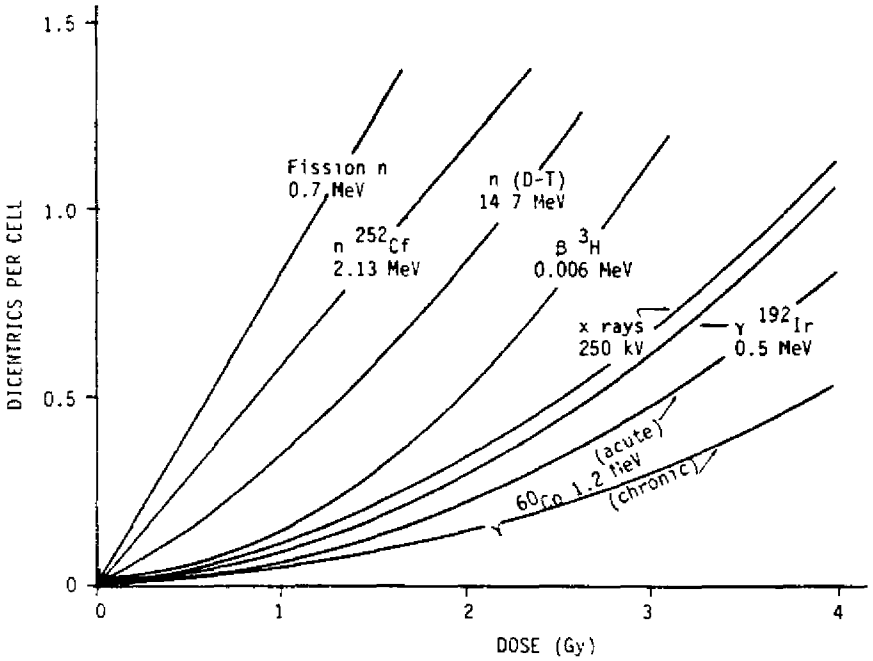

Figure XXVII. A series of generalized dose-response curves for dicentric chromosome induction for human lymphocytes irradiated in vitro. [D13]

significance [D14, J15, L15] With a few hundred cells scored from an irradiated subject, the statistical uncertainty on the dicentric yield is the main component of the 95% confidence limits on the dose estimate It is much greater than the uncertainty attached to the in vitro calibration curve [L17], so that for practical purposes the latter may be ignored when calculating confidence limits An example of an in vitro curve and the confidence limits for an exposure to gamma-radiation is given in Figure XXVIII Examples of the use of such curves in cases of accidents have been described [D15, L18]

274. For a uniform exposure to low-LET radiation, dicentrics in the scored cells follow the Poisson distribution [D13] However, in accidental irradiation, the exposure is almost always non-uniform, often involving just part of the body This results in an overdispersed distribution of aberrations The degree of departure from the Poisson distribution may be used to estimate the volume of blood exposed and its average dose [D14] This has recently been tested in an international collaborative experiment in which partial-body exposures were simulated in vitro. The resultant estimates of dose and volume irradiated were acceptably close to the true values [L28]. The calculations require a number of simplifying assumptions [I22], but they produce values that are probably more meaningful than the average whole-body dose for accidents in which clearly only part of the body has been irradiated. However, estimates of blood volume exposed may not reflect closely the proportion of body mass exposed [L39].

275 Many chromosome aberrations, including breaks and various exchanges of the chromosome or chromatid

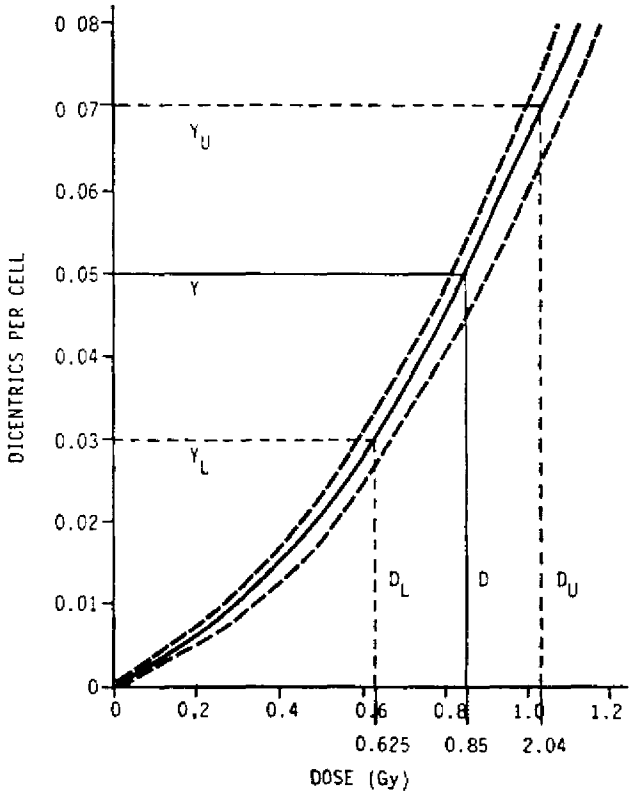

Figure XXVIII. Estimation of a dose of 0.85 Gy from a yield of 0.05 dicentrics per cell (25 in 500) by reference to an in vitro calibration curve for gamma-radiation using human lymphocytes. Statistical uncertainties on the curve are shown by the dashed curves. The upper (Y_U) and lower (Y_L) Poisson standard errors on the yield give 95% confidence limits of 1.03 and 0.625 Gy (D_U and D_L) on the dose estimate. [I22]

type, involve acentric fragments. The absence of a centromere in these fragments prevents the correct distribution of genetic material during cell division, and they are lost or incorporated in only one of two daughter cells. In the daughter cell the fragment may either join with the main nucleus or remain in the cytoplasm and form a micronucleus [K14, R19]. Recent data indicate that 20-30% of acentric fragments may become micronuclei at mitosis [B54, W23] Micronuclei may also occur as a consequence of completely aberrant configurations with more than one centromere because such structures frequently cause difficulties in chromosome separation during anaphase. They may also result from normal chromosomes which are left over because of a defect in the mitotic spindle.

276. Counting of micronuclei has been suggested as a dosimetric method for situations which include the evaluation of damage of chemical origin and the identification of particularly sensitive individuals with higher than average potential risks of developing cancer or genetic disorders. In principle, counting of micronuclei appears easier, faster and less expensive than the scoring of chromosome aberrations [I16, H33].

277. There is a higher background incidence of micronuclei than of dicentric aberrations and this may in part reflect the higher background incidence of acentric fragments due to environmental chemical mutagens. This means that the lower limit of dose detection by micronuclei is perhaps 0.25 Gy, thus making the technique less sensitive than that of scoring the dicentric yield. The frequency of micronuclei after x-irradiation of human lymphocytes in vitro reaches a peak after 96 hours of culture [F6, C29] However, by this time lymphocyte cultures have become asynchronous and individual variability in cell cycling kinetics is likely to impose considerable uncertainty in the quantification of the dose response.

278 The method can be better standardized by scoring cells which have undergone a known number of mitoses. This can be achieved by differential staining of nuclei in cells which have incorporated bromodeoxyuridine [P17], and scoring cells which have previously incorporated tritiated thymidine in S-phase or by scoring cells which have been blocked at the end of mitosis using cytochalasin B [F6].

279. Currently, the cytochalasin-B blocking method is gaining considerable popularity. This technique ensures that micronuclei are scored only in those cells that have just completed their first post-irradiation mitosis. The dose-effect relationship appears to be similar to that observed with aberrations, in that the response for low-LET radiation is quadratic and at low doses it is linear [F6, F16]. Data for exposure to high-LET radiation are not yet available. The micronucleus technique is far easier and faster than scoring for dicentrics and is more amenable to automated methods of analysis using pattern recognition systems, e.g., in polychromatic erythrocytes (PCE). Individual variability, particularly at lower doses, poses some limitations. Factors such as dose protraction, fractionation and partial-body exposure have yet to be investigated. The test can be envisaged as being particularly useful after a serious accident when many people may need to be tested quickly.

4. Dosimetry based on neurophysiological data

280. Whole-body gamma-irradiation is accompanied by immediate functional changes in the central nervous system, particularly with respect to spontaneous and evoked cerebral electrical activity. These changes have been shown in animals [B45, C30, M33] and in man [C31, C32]. They appear immediately after exposure and are an important indicator of the direct effect of radiation on the optic nerve [C31]. Radiation may also affect (a) the function of peripheral receptors; (b) nerve conduction; and (c) synaptic transmission. High doses are required to modify the function of the retina, since more than 6 Gy must be administered to cause changes in the electroretinogram. The administration of large doses (~ 10 Gy) of x or gamma rays triggers a process that lowers the excitability threshold and increases the action potential and, more irregularly, the rate of conduction of the nerve impulse. Examination of synaptic transmission produces results that are harder to interpret.

281. Irradiation, even in small doses, may cause changes in the acetylcholine-cholinesterase balance or in other chemical mediators, such as aspartic acid, adrenergic amines, and gamma-aminobutyric acid (GABA). The examination of vascular lesions makes it possible to study the role of disturbances in membrane permeability. Cell metabolism is probably disturbed, as shown by reversible changes in the nuclear chromatin. Furthermore, whole-body irradiation is accompanied by changes in the acid-base balance, essentially hypocapnia and acidosis, which can be restored from the seventh hour onwards for doses of about 1.5 Gy. It seems likely that, even if the development of functional disorders of the autonomic nervous system is not superimposable on the trend of cerebral electrical activity, changes in the acid-base balance of the blood play an important role in the genesis of the disturbances observed.

282. It has been shown by one group of investigators that whole- or partial-body gamma-irradiation of the organism can act as a stimulant or as an agent of injury, depending on the level of dose, the dose rate, the irradiated volume and, above all, the percentage of the body irradiated [C31]. There is direct stimulation of the brain and particularly of the structures in the bulbar protuberance and the hypothalamus, as well as of all the synapses in the organism. This direct stimulation is followed by an indirect stimulation of the brain by the convergence of impulses originating in the spinal cord and the bulbus in the direction of the brain. Depending on the strength of these direct or indirect stimuli and the number of impulses arising in the subcortical structures, there is an immediate defence response; the intensity and nature of this response from the central nervous system, the modifications to the autonomic nervous system and the changes in cerebral activity and behaviour will differ

with respect to both their general expression and their development These various effects combine to create an acute functional metabolic encephalopathy.

283 Disturbances in the neurophysiological equilibrium are indicated by (a) changes in excitability, consisting of successive phases of inhibition and excitation; (b) an increase in irritability in the form of paroxysmal abnormalities, ranging from a burst of slow activity through an isolated spike to a deformed spike-wave to grouped bursts of spike-waves, with rare convulsive spasms (in the case of high doses); and (c) the impossibility, at LD_{50} doses, of structures such as the hippocampus maintaining basic rhythms. On the electroencephalogram, changes in the excitation waves are noted; there is a slow-down in cerebral activity (appearance of slow, regular and broad waves), recurring spasms, slow activity or groups of slow waves, and isolated and then grouped spikes. All these phenomena are characteristic of radiation-induced effects.

284. After analysing cerebral electrical activity in the monopolar, conventional and harmonic modes, it is possible to quantify the energy changes in the power density spectrum and thus to describe objectively the slow-down in cerebral electrical activity This is achieved by calculating the extent of drift and the percentage of the recording time during which the modifications in the EEG are observed [C32]. The dose-effect relationships obtained in animals show a response above 0.25 Gy. This response appears at 15 minutes after exposure. A comparison of whole-body and head exposures makes it possible to separate the effects of direct and indirect stimulation, the latter being under the influence of the convergence of ascending impulses from the whole body. The persistence of the effect observed above 0 25 Gy during the hours following irradiation tends to indicate changes in protein synthesis and the coding of information of the neurons This dosimetric method is a valuable tool. especially if the assessment is done long after irradiation and in cases where it has not been possible to undertake chromosome analysis immediately after transfusions of blood components. The changes are persistent, particularly in cases of high doses. In survivors of doses of near the $LD_{50/60}$ the normal electroencephalographic patterns seem to take several years to reappear [C30]

5. Other dosimetric findings

285. In cases of exposure to mixed gamma-neutron fields, the dose, its neutron component and its spatial distribution can be estimated by determining the presence of ^{24}Na and ^{32}P [I9]. This radiation-induced activity can be measured in the body, blood, urine and biological or other specimens, such as hair, teeth, fingernails, clothes, metallic objects, jewellery, etc. These measurements are well standardized and form part of the physical dosimetry, in the same way as does the reconstruction of the accident. Techniques based on electron spin resonance [N7], applied to bone, hair, teeth and skin after low-LET irradiation, have shown that the signals obtained are quantifiable at lethal or sublethal doses down to about 0.3 Gy [B46, I17]. The electron spin resonance signal is stable at more than two hours after irradiation [T16, S28]. The intensity of the signal is linearly related to dose [B46, O7, I17, T16, S28]; it is greater for incident radiations of low photon energies [T16, O7], but was not detected after doses of 14 MeV neutrons [I17]. The method has been used to assess doses in accidents [S28] and in survivors from the atomic bombs and cumulative doses in occupationally exposed persons [T16].

286. Another assay described recently measures the frequency of variant erythrocytes produced by erythroid precursor cells with mutations that result in a loss of gene expression at the polymorphic glycophorin A (GPA) locus. A linear relationship was observed between variant frequency and dose received 40 years previously [L37].

287. Other techniques have also been suggested for use in biological dosimetry but have not yet been developed for man One example is cell death in hair follicles (dose-dependent from about 0 1 to 1.0 Gy) and consequent changes in hair width (dose-dependent from 1 to 10 Gy) [P10, P20]. Another is spermatogenesis, which is very sensitive to irradiation and could be used as a biological indicator of dose [H37]. DNA-synthesizing cells (spermatogonia and preleptotene spermatocytes) can be measured rapidly using flow cytometry, and their concentration in mice shows marked dose- and time-dependent changes [H37].