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CANCER MORTALITY AT A NAVAL NUCLEAR SHIPYARD

ROBERT A. RINSKY RALPH D. ZUMWALDE
RICHARD J. WAXWEILER
WILLIAM E. MURRAY, JR PHILIP J. BIERBAUM
PHILIP J. LANDRIGAN MICHAEL TERPILAK
CLINTON COX

Division of Surveillance, Hazard Evaluations, and Field Studies, National Institute for Occupational Safety and Health, Centers for Disease Control, Cincinnati, Ohio, U.S.A.; and Bureau of Radiological Health, Food and Drug Administration, Rockville, Maryland

Summary To evaluate a reported five-fold increase in leukaemia mortality among workers exposed to ionising radiation at Portsmouth (New Hampshire) Naval Shipyard (PNS), a retrospective cohort mortality study of all PNS civilian workers employed from 1952 to 1977 was done. Three subcohorts were identified: 7615 workers with radiation exposure of 0·001 to 91·414 rem (mean 2·779 rem, median 0·545 rem), 15 585 non-radiation workers, and 1345 workers selected for radiation work who received no measurable exposures. Vital status on 96% of the workers was ascertained and observed mortality due to all causes, all malignant neoplasms, and malignant neoplasms of the lymphatic and haematopoietic tissues, including leukaemia, was compared with that expected from mortality-rates for United States White males. Leukaemia mortality in radiation and non-radiation workers at PNS was also compared. Although the study had a power of greater than 99% to detect statistically a five-fold increase in leukaemia mortality among the radiation workers, and a power of 67% to detect a two-fold increase, there was no excess due to leukaemia or any other cause. The standardised mortality ratio for leukaemia among radiation workers was 84 (95% confidence interval, 34–174). There was no dose-response relation with radiation or any increased mortality in radiation over non-radiation workers. The study was, however, limited by short latency (time since first radiation); only 53% of the workers had less than 15 years' latency.

Introduction

THE carcinogenic effects of high doses of ionising radiation have been studied in radium dial painters,[1] in survivors of the atomic bombings of Japan,[2,3] in Pacific islanders exposed to atmospheric nuclear fallout,[4] in ankylosing spondylitics treated with X-rays,[5] and in children irradiated for the treatment of ringworm.[6] All these populations carried an excess risk of malignancy, particularly of leukaemia and/or cancer of the lung, thyroid, and breast. The excess leukaemia risk was evident from 2 to 30 years after exposure, and in the atomic bomb survivors was greatest at 8 years.

The carcinogenic hazard of lower doses of ionising radiation has been less clearly demonstrated. Unresolved issues include the nature of the dose-response relation at lower levels of exposure, the possible existence of a carcinogenesis threshold dose, and possible differences in carcinogenic hazard between continuous and intermittent exposure. Some recent investigations of populations exposed to low levels of ionising radiation[7–10] have, however, suggested that the risk of cancer among these groups may be greater than was previously recognised.

In May, 1978, Najarian and Colton[10] reported a five-fold increased proportional mortality due to leukaemia and a two-fold increase due to all cancer among workers who had been exposed to relatively low doses of ionising radiation while overhauling and building nuclear submarines at the Portsmouth (New Hampshire) Naval Shipyard (PNS). No increased mortality for leukaemia was observed for PNS workers not involved in nuclear work. To evaluate further this apparent excess risk of leukaemia, the National Institute for Occupational Safety and Health (NIOSH) undertook a retrospective cohort mortality study of all civilian workers at the shipyard. This study was done at the request of the Subcommittee on Health and the Environment of the United States House of Representatives. The objectives of this study were to determine whether the previously reported excess risk of leukaemia and of other cancer mortality among nuclear workers at PNS could be corroborated and to evaluate dose-response relationships between radiation and cancer.

Occupational Environment and Radiation Personnel Dosimetry

PNS is one of the oldest shipyards in the United States and was established by the British in the 1600s. In 1800, it became a U.S. Navy shipyard, with responsibility for the design, construction, and repair of naval vessels. The first U.S. submarine was built there in 1917, and the first nuclear submarine commissioned in 1958. By 1962, PNS had acquired full capability for the overhaul, repair, and refuelling of

nuclear-powered submarines. Sixty-three nuclear submarines had been constructed, overhauled, or repaired at PNS by 1977. The trades represented at PNS have included forging, machining, painting, welding, and metal fabricating. PNS is unique among U.S. Navy shipyards in that it has the longest history of nuclear work, and that most of the work done within the past thirty years has been limited to the construction and overhaul of submarines, both diesel and nuclear powered.

Since the beginning of the Navy nuclear propulsion programme in the 1950s, certain workers at PNS have been selected and trained for work in limited-access radiation-controlled areas on the basis of their special skills in particular trades. All areas where potential radiation exposures existed were designated radiation-controlled areas. Before any workers could become eligible to work in radiation-controlled areas, they were given a special medical examination. This examination was repeated every 3 years. Since 1972 (earliest date for which these records exist), 89 persons have been removed from the nuclear programme on the basis of findings in the repeat medical examination; the number excluded on the basis of the initial examination findings is not known.

Candidates for radiation work at PNS were required to complete successfully a radiation safety training programme. Such workers were deemed "qualified" and given a personnel dosimeter when access to radiation-controlled areas was necessary. All workers in radiation-controlled areas were required to wear dosimeters.

Monitoring of workers at PNS for external radiation exposure began in 1950 with the introduction of a film badge programme for industrial radiographers, a small group of workers engaged in the X-ray examination of the integrity of welds. The programme was expanded when nuclear work began in 1958, and has continually been refined. Among the refinements was the replacement of film badges by calcium fluoride thermoluminescent dosimeters in 1974. Each worker's external radiation dose was entered into his personal dose file. Radiation exposure from internal radioactivity (inhaled, ingested, or absorbed radionuclides) also has been recorded. There have, however, been no instances recorded at PNS in which internal radioactivity has resulted in exposure of a worker to greater than 10% of the allowed annual Federal limit for occupational radiation exposure. On that basis, only external exposures were used for dose calculations in this study.

In addition to possible radiation exposure, workers were exposed to other airborne contaminants. Toxic materials such as carbon tetrachloride, tetrachlorethane, and benzene had been used at the shipyard, but during the past decade they have been eliminated or replaced with less toxic substitutes. Asbestos use at PNS has also been reduced.

Methods

The study cohort consisted of all White males ever employed at PNS between Jan. 1, 1952, and Aug. 15, 1977. Race was unknown for most PNS employees, but because almost all past PNS employees were believed to have been White, we assumed all persons of unknown race to be White. We excluded known non-Whites from analysis because of their small numbers; women were excluded since, historically, they were not involved in production work. We chose the starting date as Jan. 1, 1952, because complete personnel records exist only since then. The study end-date of Aug. 15, 1977, represented the last date through which vital status was ascertained; in our analysis, we considered any persons who died after that date to be alive. Persons whose vital status could not be determined (lost to follow-up) were considered to have been alive through to the end of the study. This procedure slightly underestimates mortality. A total of 24 545 workers fit the cohort definition.

We calculated each worker's total external radiation dose from a summary computer printout of individual annual radiation exposures, provided by PNS. To determine the accuracy of this summary printout, we selected a random sample of workers and verified their reported radiation doses against the original dose file. Recorded doses accurately reflected original dose data.

We divided the total cohort into three subcohorts, on the basis of radiation exposure, as follows:

Subcohort I (exposed radiation workers).—7615 White males who had worked at least 1 day at the shipyard between Jan. 1, 1952, and Aug. 15, 1977, and who had a recorded lifetime cumulative radiation exposure of at least 1 mrem.

Subcohort II (non-radiation workers).—15 585 White males who had also worked between Jan. 1, 1952, and Aug. 15, 1977, but who had no record of ever having been assigned to radiation areas.

Subcohort III (unexposed radiation workers).— 1345 White males who had worked at the shipyard between Jan. 1, 1952, and Aug. 15, 1977, and who were qualified for radiation work and monitored, but whose total lifetime cumulative dose was 0·000 rem. This subcohort was not completely identified, because the shipyard did not record doses of 0·000 rem during certain years. Our examination of mortality in this group was intended to evaluate the possibility that there may have existed systematic differences between radiation-qualified and non-radiation workers, apart from any differences resulting from exposure to radiation.

Death certificates were obtained for deceased individuals and were coded by a nosologist according to the revision of the International Classification of Diseases (Adapted) in effect at the time of death.

For the total cohort and for all subcohorts, we used a NIOSH life-table analysis system to generate expected numbers of cause-specific deaths. These numbers of deaths are based on United States White male death rates by 5-year age groups and 5-year calendar time periods. Expected and observed deaths were accumulated for each age group and calendar time period from Jan. 1, 1952, through to Aug. 15, 1977. Observed and expected deaths were stratified by 5-year latency (time since initial PNS employment) periods and by 5-year periods of duration of employment.

For subcohort I, we stratified observed and expected deaths by latency (time since initial external radiation exposure of at least 1 mrem) and also by cumulative lifetime radiation dose category. The dose categories, which we chose before data analysis, were 1–29, 30–99, 100–499, 500–999 mrem, and then 1·000–4·999 rem, 5·000–14·999 rem, and 15·000 rem and over. Because only annual radiation doses were used, we assumed each person to have received that dose at a constant rate throughout the year. As a person accumulated increasing dose, his person-years-at-risk were allocated to the corresponding dose categories.

For the total cohort and for subcohort II, person-years-at-risk began on the first date of employment at PNS or on Jan. 1, 1952, whichever was later. In subcohort I, person-years-at-risk began on Jan. 1, 1952, for persons with radiation exposures prior to that date. For other persons in subcohort I, person-years-at-risk began on the first date of employment in the first year in which the person's cumulative radiation dose was greater than or equal to 1 mrem. This procedure added an average of 6 extra months of observation per person, and thus tended slightly to overestimate time at risk. In subcohort III, person-years-at-risk began on the first date of employment in the first year in which the person's recorded cumulative dose was 0·000 rem. In all cohorts, person-years-at-risk ended on the date of death or on Aug. 15, 1977, whichever came first. We calculated standardised mortality ratios (SMR = observed deaths/expected deaths × 100) and their 95% confidence intervals.[11] When the confidence interval indicated statistical significance, we calculated exact p values assuming the Poisson distribution.

To permit an intra-cohort comparison of leukaemia mortality in radiation and non-radiation workers, we calculated 10-year age-specific and 13-year calendar time-specific leukaemia mortality rates for subcohort II; those rates were then applied to the person-

years-at-risk in subcohort I to derive an expected number of deaths. An SMR was then calculated. The age-time intervals indicated were chosen to create categories with enough person-years to generate reasonably stable rates.

Results

We found that 18 771 workers (76%) of the total cohort were alive, 4762 (19%) had died, and 1012 (4%) were lost to follow-up (table I). There were 4762 deaths observed, whereas 5361 were expected ($p<0\cdot001$) (table II).

TABLE I—VITAL STATUS, AS OF AUG. 15, 1977, FOR WHITE MALES EVER EMPLOYED AT PORTSMOUTH NAVAL SHIPYARD FROM JAN 1, 1952

Status	Total	Subcohort I: lifetime radiation ≥1 m rem	Subcohort II: non-radiation	Subcohort III: lifetime radiation 0·000 rem
Alive	18 771 *(76%)*	6658 *(87%)*	10 999 *(71%)*	1114 *(83%)*
Dead	4762 *(19%)*	833 *(11%)*	3733 *(24%)*	196 *(15%)*
(death certificates obtained)	(4401) *(92%)*	(797) *(96%)*	(3419) *(92%)*	(185) *(94%)*
Lost to follow-up	1012 *(4%)*	124 *(2%)*	853 *(5·5%)*	35 *(3%)*
Total	24 545 *(100%*)*	7615 *(100%*)*	15 585 *(100%*)*	1345 *(100%*)*

*Totals may not equal 100% due to rounding

TABLE II—OBSERVED AND EXPECTED DEATHS AMONG 24 545 WHITE MALES (TOTAL COHORT)

Cause of death*	Observed	Expected	SMR	95% confidence interval for SMR
All causes	4762	5361	89	86–91
All malignant neoplasms (140–207)	977	1032·8	94	89–101
Leukaemia (204–207)	39	41·5	94	67–128
All neoplasms of lymphatic and haematopoietic tissue (200–207)	84	99·1	85	68–105

*International Classification of Diseases (Adapted) (8th revision).

Deaths due to leukaemia, to all lymphatic and haematopoietic neoplasms, and to all malignant neoplasms were slightly lower in the total cohort than expected. For each of the causes of death examined in the total cohort, no trends in risk were noted either by latency or by duration of employment.

In subcohort I (exposed radiation workers), cumulative lifetime radiation doses ranged from 0·001 to 91·414 rem (mean 2·779 rem, median 0·545 rem). Mortality from all causes combined in this subcohort was significantly below expected ($p<0\cdot001$) (table III). Also, mortality rates for all specific causes were less than expected, although none was significantly depressed. Of the 7 leukaemia deaths observed in subcohort I, based on death certificate diagnoses and available medical records, 2 were lymphatic, 2 chronic myelogenous, 1 acute, and 2 were unspecified as to type.

In subcohort II (non-radiation workers), observed mortality was approximately as expected for all causes of death combined, as well as for each specific cause (table IV). Risks for specific causes tended to be slightly higher in subcohort II than in subcohort I.

In subcohort III, total and cause-specific SMRs were lower than for either of the other subcohorts (table V). Mortality from all causes of death combined was significantly depressed ($p<0\cdot001$).

TABLE III—OBSERVED AND EXPECTED DEATHS AMONG 7615 WHITE MALES WITH RECORDED LIFETIME CUMULATIVE RADIATION DOSE GREATER THAN OR EQUAL TO 0·001 REM (SUBCOHORT I)

Cause of death	Observed	Expected	SMR	95% confidence interval for SMR
All causes	833	1065·3	78	73–84
All malignant neoplasms (140–207)	201	218·5	92	80–106
Leukaemia (204–207)	7	8·3	84	34–174
All neoplasms of lymphatic and haematopoietic tissue (200–207)	15	21·1	71	40–117

TABLE IV—OBSERVED AND EXPECTED DEATHS AMONG 15 585 WHITE MALES WITH NO RECORDED HISTORY OF RADIATION EXPOSURE (SUBCOHORT II)

Cause of death	Observed	Expected	SMR	95% confidence interval for SMR
All causes	3733	3801·0	98	95–101
All malignant neoplasms (140–207)	726	723·6	100	93–108
Leukaemia (204–207)	31	29·1	106	72–151
All neoplasms of lymphatic and haematopoietic tissue (200–207)	67	67·7	99	77–126

In none of the cohorts did we observe any trends by 5-year calendar time periods or by 5-year age groups in mortality rates for any malignant neoplasms of the lymphatic and haematopoietic tissues. Also, we noted no significant excesses or trends in SMRs for non-malignant diseases of the blood-forming organs. Finally, we observed no trends in mortality when malignant and non-malignant haematological diseases were combined and examined together. In subcohort I, we found no significant trends by radiation dose for mortality from malignant neoplasms of the lymphatic and haematopoietic tissues or for all malignancies combined (table VI).

In comparing leukaemia mortality between radiation (subcohort I) and non-radiation (subcohort II) workers, we found that 10·05 leukaemia deaths would have been expected in subcohort I based upon the age- and calendar-specific rates of leukaemia experienced by subcohort II. However, only 7 deaths due to leukaemia were observed in subcohort I (SMR = 70); this difference was not statistically significant.

TABLE V—OBSERVED AND EXPECTED DEATHS AMONG 1345 WHITE MALES WITH RECORDED LIFETIME CUMULATIVE RADIATION DOSE = 0·000 REM (SUBCOHORT III)

Cause of death	Observed	Expected	SMR	95% confidence interval for SMR
All causes	196	298·9	65	57–75
All malignant neoplasms (140–207)	50	59·5	84	62–111
Leukaemia (204–207)	1	2·3	*	*
All neoplasms of lymphatic and haematopoietic tissue (200–207)	2	5·75	*	*

*For causes with less than 6 expected or observed deaths, no SMRs or confidence intervals were calculated.

TABLE VI—OBSERVED (O) AND EXPECTED (E) DEATHS FOR 7615 WHITE MALES (SUBCOHORT I) WITH CUMULATIVE LIFETIME RADIATION DOSE ≥0·001 REM, BY SPECIFIC DOSE CATEGORIES

Cause of death		Dose (rem) 0·001–0·029	0·030–0·099	0·100–0·499	0·500–0·999	1·000–4·999	5·000–14·999	15·000 and over	Total
Hodgkin's disease	O	0	0	2	0	1	0	0	3
	E	0·4	0·5	0·7	0·3	0·6	0·2	0·1	2·9
Lymphosarcoma and reticulosarcoma	O	0	0	1	0	2	0	0	3
	E	1·0	1·1	1·7	0·7	1·4	0·6	0·2	6·8
Leukaemia and aleukaemia	O	1	0	1	1	3	0	1	7
	E	1·3	1·4	2·1	0·9	1·6	0·7	0·2	8·3
Other neoplasms of lymphatic and haematopoietic tissue	O	1	0	0	0	1	0	0	2
	E	0·5	0·5	0·8	0·3	0·6	0·2	0·1	3·1
All malignant neoplasms	O	29	32	46	26	45	17	6	201
	E	33·2	37·1	56·8	23·5	42·8	18·0	7·2	218·5
Person-years-at-risk		14 810	15 960	24 138	10 418	21 769	8350	2778	98 223

Discussion

This study had a greater than 99% probability of detecting statistically the five-fold increased risk of leukaemia mortality reported by Najarian and Colton[10] among radiation-exposed workers at PNS. Further, had the true relative-risk of death from leukaemia at PNS been 2·2, the likelihood of our having detected such a risk would still have been 80%. However, when we compared mortality in the shipyard workers with death rates of the United States White male population, we found no excess. Also, we found no leukaemia excess when we compared radiation workers at PNS with an internal control—the non-radiation workers at the same shipyard. Finally, in the PNS radiation workers, we found no positive dose-response relationships between ionising radiation dose and mortality from any cause reported.

The difference between our results and those of Najarian and Colton[10] seem to reflect our more complete ascertainment of the PNS cohort, thus avoiding selection bias, and our more accurate classification of workers as to their radiation exposure. Our results are, in large part, corroborated in a reanalysis of the data of Najarian and Colton[10] which was undertaken by Prof. T. Colton and others (unpublished) after they had received more accurate nosology and radiation dose information on PNS workers.

Our finding of a deficit in mortality in this worker population is not surprising and can in all likelihood be attributed to the "healthy worker effect".[12] This happens when a standard population, such as all United States White males, is used to generate expected numbers of deaths for an employee population. Most populations of workers are healthier than the general population, because the latter includes numbers of chronically ill or otherwise unemployable persons. The healthy worker effect is, however, not believed substantially to affect deaths due to malignant neoplasms. The PNS radiation workers demonstrated a particularly strong healthy worker effect, most likely due to the special medical screening which they underwent before acceptance into the radiation programme.

Despite the apparently negative findings of the present study, it would be quite improper to interpret our results as constituting evidence against the leukaemogenesis of low doses of external ionising radiation. Our study has substantial limitations which preclude that conclusion. One limitation is that an insufficient number of years may have elapsed since initial exposure for a large number of the radiation workers at PNS to permit the manifestation of cancers requiring long latency. 20% of PNS radiation workers were first irradiated only after 1967, and thus had less than 10 years' latency; only 1% of the radiation workers had 25 or more years' latency. In addition, the number of workers with radiation exposure was small. Thus, the likelihood of our having observed a slight excess in mortality is correspondingly small. If, for example, the true relative risk of leukaemia mortality in radiation workers at PNS were 1·1, as might be suggested by extrapolation from high-dose studies,[13] our study would have had only a 5% probability of detecting such a risk; if the true relative risk were 1·5, the likelihood of detection would have been 25%.

Another limitation is that our ascertainment of vital status was only 96% complete. While this percentage of follow-up is well within the range generally considered acceptable for cohort mortality studies, the 4% deficit had the effect of inflating person-years-at-risk and of slightly lowering SMRs. Our failure to obtain certificates of death on 8% (361) of the known deceased workers further reduced slightly the true SMR for each specific cause of death.

Our use of the non-radiation workers as an internal comparison population for calculating expected leukaemia deaths for the radiation workers was intended to hold constant any geographic or demographic differences that may have existed in the external comparison between PNS workers and all United States White males. This internal comparison did not, however, account for the possible selection of particularly healthy individuals for radiation work. Thus, the SMR of 70 for leukaemia for subcohort I as compared to subcohort II should be interpreted with caution.

We undertook this study in 1978 to satisfy the urgent and valid requirement of workers, the public, and the United States Congress for accurate, current information on the cancer risk of occupational exposure to low levels of ionising radiation. Although we saw no excess cancer, it is possible that a pattern of excess cancer mortality may become manifest at PNS after another 10–20 years. The study has served to establish for further study a relatively large cohort of workers with known radiation dose. Follow-up analysis of the cohort will be undertaken.

Effects due to radiation are not limited to cancer, and events other than death may occur. Such effects are beyond the scope of this study, but might include induction of chromosome aberrations[14] or of recessive mutations in somatic or germinal cells which might become evident only generations hence. Only time and further studies will allow assessment of such additional hazards of chronic exposure to low doses of ionising radiation.

Requests for reprints should be addressed to R. A. R., N.I.O.S.H., 4676 Columbia Parkway, Cincinnati, Ohio 45226, U.S.A.

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LONG-TERM IMPAIRMENT OF SUPPRESSOR-CELL FUNCTION BY CYCLOPHOSPHAMIDE IN MINIMAL-CHANGE NEPHROPATHY AND ITS ASSOCIATION WITH THERAPEUTIC RESPONSE

D. TAUBE Z. BROWN
D. GWYN WILLIAMS

Renal Unit, Department of Medicine, Guy's Hospital, London SE1

Summary Lymphocyte suppressor-cell function was studied by induction with concanavalin A in 31 patients with minimal-change nephropathy (MCN) in remission. 21 patients had been treated with cyclophosphamide 0·5–12·0 years previously (mean 6·5 years) and had been in remission for 0·5–9·0 years (mean 5·1 years). The remaining 10 patients had never received cyclophosphamide and had been in remission for 1–10 years (mean 5·3 years). The cyclophosphamide-treated group had significantly less suppressor-cell function than either the controls or the non-cyclophosphamide-treated group, the latter being not significantly different from normal. When patients who had received cyclophosphamide were divided into those who had relapsed after taking this drug (10 patients) and those who had not (11 patients), suppressor-cell function was significantly impaired in the non-relapsing group. This association of impaired suppressor-cell function with failure to relapse may indicate that suppressor cells have a pathogenetic role in MCN and that the therapeutic effect of cyclophosphamide in this disease is to diminish their function. Alternatively, the impaired suppressor-cell function in the non-relapsing group may be simply a marker of effective treatment with cyclophosphamide. The finding of long-term suppression of lymphocyte function after cyclophosphamide coupled with this drug's risks of causing malignancy and gonadal dysfunction reinforces the need for caution in its use in MCN.

Introduction

CONSIDERABLE circumstantial evidence implicates immunological mechanisms in the pathogenesis of minimal-change nephropathy (MCN).[1] In particular, the response of this disease to corticosteroids and/or cyclophosphamide has been used to support the hypothesis that MCN is caused by a disorder of T-cell function.[2] Although of undoubted efficacy, the mode of action of these drugs in MCN is unknown. We now present evidence that cyclophosphamide produces long-term impairment of suppressor-cell function in patients with MCN in remission and that this phenomenon is associated with protection against subsequent relapse of their disease.

Subjects

The diagnosis of MCN in the 31 patients studied had been confirmed histologically at the time of their presentation. All patients were in remission—i.e., with no proteinuria, normal serum-albumin levels, and normal renal function. Suppressor-cell function was studied in 21 patients who had been treated with cyclophosphamide 0·5–12·0 years previously (mean 6·5 years) and in 10 patients who had not received this drug. The age, period of current remission, dosage of cyclophosphamide, and relapse after cyclophosphamide therapy of these patients are shown in the table. The average age of the cyclophosphamide-treated patients was 32·1 years and the non-cyclophosphamide-treated patients 41·2 years. The cyclophosphamide-treated patients had been in remission for an average period of 5·1 years and the non-cyclophosphamide-treated patients 5·3 years. All the cyclophosphamide-treated patients and 9 of the 10 non-cyclophosphamide-treated patients had received corticosteroids in the past; the exception, no. 27, had remitted without treatment. In each case corticosteroid therapy had been stopped shortly after the patient had entered remission.

Supressor-cell function was also studied in 30 healthy control subjects (17 men, 13 women) average age 30·2 years (range 18–52). None of the patients or controls were taking any medication known to alter suppressor-cell function or mitogen-induced lymphocyte transformation.

Methods

Peripheral-blood lymphocytes were separated from venous blood with 'Ficoll' (Pharmacia)/'Triosil' (Nyegaard) gradients, washed, and adjusted to a concentration of 4×10^6 cells/ml in buffered tissue-culture medium (TCM) containing fetal-calf serum 10% and gentamicin 5 μg/ml. The cell suspension was divided into two parts, one being designated responder (R) cells and the other suppressor (S) cells. Concanavalin A (CON A) (Miles Yeda) at a final concentration of 5 μg/ml was added to the S cells. Both suspensions were then incubated at 37°C for 24 h. The S cells were then resuspended and thoroughly washed in TCM to remove any unbound CON A and adjusted to 4×10^6 cells/ml. 100 μl volumes of similarly washed and adjusted R cells were added to 100 μl of S cells (RS), mixed with 400 μl TCM, and stimulated with 200 μl CON A at final concentrations of 2, 4, 6, and 8 μg/ml. Separate cultures of 100 μl R and 100 μl S cells were added to TCM and CON A as above. Unstimulated control cultures for RS (x_1), R (x_2), and S (x_3) consisted of mixtures as above but without CON A. 200 μl volumes of these cultures were then incubated at 37°C in triplicate in microtitre plates, pulsed at 48 h with 0·3 μC tritiated thymidine, and harvested onto glass-fibre filters 24 h later. The incorporated radioactivity expressed as disintegrations per minute (dpm) was measured in a β-counter.