Phenylbutazone and chromosomal damage

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Walker, S., Price Evans, D. A., Benn, P. A., Littler, T. R., and Halliday, L. D. C. (1975). *Annals of the Rheumatic Diseases*, 34, 409–415. **Phenylbutazone and chromosomal damage.** Investigation of 44 paired test and control patients, all suffering from rheumatoid arthritis, following exposure to phenylbutazone (PBZ) and/or oxyphenbutazone (OPB), suggests that there is no significant increase in the level of chromosomal damage in lymphocytes. The control subjects comprised two series, one previously exposed to PBZ and/or OPB, but not for at least 1·5 years, and the other never exposed to PBZ or OPB. No significant difference in the level of chromosome damage was found between patients never exposed, previously exposed, or now receiving PBZ and/or OPB.

The importance of studies to evaluate the possible genetic effects of commonly used drugs cannot be overemphasized. The occurrence of aplastic anaemia, hypoplastic anaemia, and pancytopenia have been frequently reported (Committee on Safety of Medicines, 1972) in patients receiving phenylbutazone therapy. The findings of Stevenson, Bedford, Hill, and Hill (1971) of increased chromosomal damage in patients taking phenylbutazone (PBZ), and the report by Wissmüller (1971) of chromosomal damage induced by PBZ in human lymphocytes *in vitro* were therefore particularly relevant to such studies and indicated the necessity for further investigation.

Stevenson and others (1971) reported a significantly higher level of chromosomal damage in patients suffering from rheumatic disorders who had taken PBZ over a period of at least 6 weeks than in controls. Investigations of a similar nature on horses treated with PBZ (Stevenson, Hastie, and Archer, 1972) did not show any significant difference compared with a series of control animals.

In order to provide further data on the cytogenetic effects of PBZ, patients from the Liverpool Regional Rheumatology Centre at Leasowe Hospital were investigated (1) for chromosomal damage in lymphocytes and (2) for DNA synthesis in the marrow cells. This paper reports our findings after the short-term culture of lymphocytes from patients suffering from rheumatoid arthritis. The study of marrow specimens is still in progress.

Patients

Studies were made during 1972 and 1973 on 88 patients (62 female and 26 male), all having rheumatoid arthritis as

defined by the American Rheumatism Association (1959) criteria. Of the 44 test patients 36 were currently receiving PBZ and 8 were receiving oxyphenbutazone (OPB). Each test patient was paired with a control patient, matched by age (within 5 years), sex, and social class. It is difficult to find patients with rheumatoid arthritis who have never received PBZ or OPB. Hence the initial criteria for a control patient was one who had not received either drug for at least 12 months and preferably never.

After all the data had been accumulated the control patients were separated into two categories: (1) those who had never received PBZ or OPB, and (2) those who had received either in the past but not for at least 12 months. Therefore two effective 'control' series, each paired with test patients, were available for comparisons within the population sample. Twenty-two control patients had received PBZ in the past for varying periods of a few weeks to years, but this therapy had been terminated for at least 1·5 years before blood for this study was taken. Three of these 22 had also received OPB but not for at least 3 years. A further three controls had received OPB (never PBZ), but had discontinued it at least 2 years before the study. The remaining nineteen control patients were completely unexposed to these agents.

Experimental observations

CLINICAL DATA

The following items were noted for each patient. (i) Age. (ii) Social class according to General Register Office (1960) criteria. No grouping was possible on three test patients, Cases 17, 19, and 22. (iii) Functional grade of rheumatoid arthritis (Steinbrocker, Traeger, Batterman, 1949). (iv) Duration of rheumatoid arthritis. (v) Present medications with duration of exposure and the weight estimated to have been consumed. In two test patients conservative estimates of PBZ consumption had to be made in the pre-

sence of inadequate medication records (Case 10, 1000 g; Case 15, 400 g). No estimate could be made for Case 37. (vi) Previous medications with duration of exposure, but no accurate estimation of the weight consumed was possible. This applies also to the past consumption of PBZ and OPB by control patients. (vii) As an index of irradiation the x-ray envelope was weighed. This was considered admissible because the patients had not been exposed to x-rays in other hospitals to any significant degree. In six instances the actual x-rays were not available so a 'duplicate' set was made up and weighed. (viii) Most recent recorded rheumatoid arthritis latex agglutination test results, taking a titre when available.

INVESTIGATION OF BLOOD SAMPLES

(i) Plasma PBZ concentration was estimated by the method of Burns, Rose, Chenkin, Goldman, Schulert, and Brodie (1953). For this purpose the blood was anticoagulated with heparin and kept at +4°C for a maximum of 10 days before analysis. The specimens were presented 'blind' to the analyst. Eight specimens from control patients had apparent concentrations of 5 µg/ml per ml or less, which is within the 'blank' value for the method. The eight specimens from test patients taking OPB gave apparent PBZ concentrations between 0 and 18 µg/ml. The analytical methods for OPB and PBZ are different but high concentrations of OPB will give apparent values higher than blank in the PBZ assay. (ii) Haemoglobin concentrations, and (iii) total leucocyte counts were both made by conventional techniques using a Coulter counter. (iv) Erythrocyte sedimentation rate (ESR) was estimated by the Westergren technique.

CYTOGENETIC INVESTIGATIONS

Blood from patients was cultured for 48 hours following a modification of the technique of Arakaki and Sparkes (1963). Colchicine at a final concentration of 0.0002% for metaphase arrest (1 h) and 0.075 mol/l KCl hypotonic treatment (10 min) were used. Slides were prepared by the standard air drying method and stained in lactic-aceto-orcein.

In order to maintain a constant standard, one of us (P.A.B.) was entirely responsible for the blood cultures, slide preparation, and scoring of chromosomal damage, without prior knowledge of which specimens referred to control or test patients. All types of damage were scored, including single chromatid gap or break (B), unstable chromosome arrangements (Cu), and stable chromosome arrangements (Cs). Ring (R) and dicentric (D) chromosomes were particularly noted. Cells were not fully analysed, however, and hence the level of Cs anomalies may be slightly low owing to minor subtle changes not being recognized.

In test Cases 18, 19, 20, and 23, the number of cells scored were 26, 39, 50, and 63, respectively. In all other cases 100 cells were scored for chromosomal damage.

Results

The test and control patients were similar in age, socioeconomic status, exposure to diagnostic x-rays, rheumatoid arthritis latex titre, haemoglobin concentration, total leucocyte count, and ESR as shown by Wilcoxon's signed ranks test of significance (Langley, 1970) in Table I (the correction given by

Table I Patient data

Measurement	Test patients			Control patients			Wilcoxon's signed ranks test			
	No.	Mean	SD	No.	Mean	SD	No. of usable pairs	Lowest R value	z	P
Age (years)	44	55.4	9.7	44	55.0	9.5	39	351	0.54	>0.10
Social class	41	2.99	0.99	44	3.02	0.85	14	63.4	—	>0.10
Functional grade of rheumatoid arthritis	44	2.25	0.51	44	2.16	0.41	12	13	—	<0.05
Weight of x-ray envelope (oz)	44	18.95	9.75	44	16.14	10.94	42	360	1.14	>0.10
Reciprocal of rheumatoid arthritis latex titre	42	149.5	230.6	43	145.6	178.5	23	130	0.23	>0.10
Haemoglobin (g/dl)										
Males	13	13.69	1.52	13	13.08	1.65	13	36.5	—	>0.10
Females	31	12.22	1.58	31	12.50	1.38	30	174.5	1.18	>0.10
Log ₁₀ total leucocyte count (×10 ⁻³)	44	0.8786 (7.561)	0.1200 (1.318)	44	0.8977 (7.902)	0.1110 (1.291)	44	433	0.72	>0.10
ESR (mm/h)	44	40.89	33.40	44	48.23	32.25	42	368.5	1.03	>0.10
Log ₁₀ duration of rheumatoid arthritis (m)	44	2.0665 (116.5)	0.2863 (1.933)	44	1.7156 (51.95)	0.4635 (2.907)	43	172.5	3.62	<0.002

Where the data were log normal, antilog figures are given in parentheses and the logarithms were used in the signed ranks test.

Armitage (1971) was employed for the computation of z). There was, however, a significant difference between them in that the duration of rheumatoid arthritis was longer in test patients ($P < 0.002$) and the functional grade of test patient was also greater than that of controls ($P < 0.05$).

Controls who had been exposed to PBZ (and/or OPB) were compared with those never exposed for each of the measurements shown in Table I. Wilcoxon's sum of ranks test was used but no significant difference was shown ($P > 0.10$) except in one instance, namely, the $\log_{10}$ duration of rheumatoid arthritis. Here $z = 4.19$ and $P < 0.002$. The mean for those exposed was 1.9711 (anti-log 93.56) and the range 27–516 months. Whereas for the unexposed the mean was 1.3792 (anti-log 23.94) with a range of 4–72 months.

The cumulative dose of PBZ in the test cases was log-normally distributed with a mean of 2.54 (anti-log, 347) $\pm$ 0.55 SD (anti-log, 35) and a range of 6–1500 g. For this calculation the weights of OPB were tested in the same manner as PBZ. The mean plasma PBZ concentration in 36 test subjects receiving PBZ (but excluding 8 on OPB) was 81.86 $\pm$ 29.91 SD $\mu\text{g/ml}$ (range of 25–149 $\mu\text{g/ml}$).

Both test and control patients had in the past received and/or were receiving at the time of study a

selection from 61 medications other than PBZ or OPB (Tables II and III).

The data on chromosomal damage found in all patients are summarized in Tables IV and V and analysed in Table VI. So as to give an indication of the total damage seen after short-term culture, type B damage cells have also been included in some of the calculations. However, the most relevant comparisons are those using the data for cells showing Cu, Cs, R, or D chromosomal arrangements as these are an indication of damage *in vivo* (before DNA synthesis *in vitro*). In order to detect any differing trends in the distribution of chromosomal damage, use of Wilcoxon's distribution-free statistical tests was made whenever possible. Yates's χ^2 and Fisher's exact tests were applied in the analysis of presence or absence of R + D in patients.

Comparison of the percentage damage scored as Cu + Cs or R + D indicates somewhat raised values for the test patients and previously exposed controls, relative to controls never exposed to PBZ or OPB (Table V). However, these are not significantly different as shown by the detailed analysis in Table VI.

There was close correlation in the percentage damage between the 44 pairs of test and control patients (including controls previously exposed to PBZ or OPB) and also between the 19 pairs of test

Table II Index of drugs

PBZ	Phenylbutazone	31	Phenobarbitone
OPB	Oxyphenbutazone	32	Imipramine
1	Prednisolone	33	Prochlorperazine
2	Cortisone	34	Amitriptyline and diazepam
3	Betamethasone	35	Phenobarbital and theobromine
4	ACTH	36	Fenfluramine
5	Pentazocine	37	Amitriptyline
6	Aspirin	38	Chlorpromazine
7	Indomethacin	39	Protriptyline
8	Benorylate	40	Penicillamine
9	Paracetamol	41	Methandienone
10	Ibuprofen	42	Stanozolol
11	Alclofenac	43	Nandrolone phenylpropionate
12	Flufenamic acid	44	Chlorpropamide
13	Chloroquine	45	Ascorbic acid
14	Hydroxychloroquine	46	Thyroxine
15	Dextropropoxyphene	47	Slow K
16	Disprin	48	Frusemide
17	Mefenamic acid	49	Bendroflumazide
18	Aloxiprin	50	Digoxin
19	Ibufenac	51	Chlorpheniramine
20	Nifenazone	52	Polymethylsiloxane
21	Dihydrocodeine	53	Aluminium hydroxide gel
22	Paracetamol and dextropropoxyphene	54	Propantheline
23	Aspirin and codeine	55	Streptomycin
24	Ethioheptazine and aspirin	56	Proxiphylline
25	Sodium aurothiomalate	57	Aminophylline suppositories
26	Iron dextran injection	58	Bromhexine hydrochloride
27	Oral iron preparations	59	Franol
28	Chlordiazepoxide	60	α Methyl dopa
29	Nitrazepam	61	Trypsin
30	Diazepam		

Table III Drug medications in paired patients

Patient pair no.	Test patients		Control patients	
	Past therapy	Present therapy	Past therapy	Present therapy
1	PBZ 1, 25	PBZ 6	7, 10, 18, 25	7, 18
2	PBZ 6, 7, 25, 27, 31, 45	PBZ 6, 7, 29	1, 7, 18, 22, 25, 26, 46	1, 7, 46
3	PBZ 1, 6, 9, 18, 35, 47, 48	PBZ	PBZ (1964) 1, 4, 25, 43, 47, 48	7, 22, 53
4	PBZ 22, 25, 26, 27	PBZ 1, 22	PBZ (1971) 1, 7, 25, 30, 32	1, 25, 32
5	PBZ 1, 7, 14	PBZ 22	PBZ (1969) 6, 9, 25	NIL
6	PBZ 9, 22, 25, 27, 29	PBZ 9, 27	7, 18, 51	6
7	PBZ 6	PBZ 6	PBZ (1970) 6 (15 + 6) 7, 22	(15 + 6) 32
8	PBZ 37	PBZ	OPB (1966) 1, 9, 22, 25	1
9	PBZ OPB 1, 7, 22	PBZ OPB	25, 18	18
10	PBZ 7, 14, 16, 25	PBZ	6, 7, 9, 10, 25	10
11	PBZ 1, 60	PBZ 1, 60	7, 10	7, 18, 30
12	PBZ 6, 14, 25	PBZ	7 (6 + 9) 9, 10, 18, 22, 25	7, 18, 22
13	PBZ 18, 22, 25	PBZ	PBZ (1961) 1, 6, 7, 25, 32	1
14	OPB 1, 7, 14, 22, 25, 28, 32, 52	OPB 1	PBZ (1970) 7, 9, 18, 22, 30, 33, 34, 47, 48, 50	10
15	PBZ (PBZ + 1) 1, 3, 7, 10, 25, 32	PBZ 7, 22, 37, 38	7, 18, 22, 25, 47, 49	7, 18
16	PBZ (PBZ + 1)	PBZ 1	'steroids' 7, 10, 22, 29, 30	7, 11
17	PBZ (PBZ + 1) 1, 2, 6, 14, 22, 25	PBZ	PBZ (1970) 1, 6, 7, 22, 25, 27, 32	1
18	PBZ	PBZ	7, 18, 25	7, 18, 25
19	PBZ 14, 27	PBZ 27	PBZ (1964) 1, 7, 10, 16, 25, 28, 42	1
20	PBZ 7, 22, 25	PBZ 27	PBZ (1965) 1, 2, 17, 25, 44	1, 22, 44
21	PBZ 25	PBZ 25	OPB (1970) 7, 10, 18, 25	16
22	OPB 3, 25	OPB 24, 53	PBZ (1971) 2, 7, 16	7, 18
23	PBZ 7, 18, 25	PBZ 7, 25	OPB (1969) (PBZ + 9) (1969) 22, 28, 54	22
24	PBZ 18, 27, 45	PBZ	7, 25	7
25	OPB 6, 25	OPB 9	PBZ (1971) 1, 11, 13, 25	11
26	PBZ 18, 19, 21, 28	PBZ	7, 18, 22	7, 18, 22
27	PBZ OPB 1, 7, 16, 22, 25, 30, 40	OPB 1, 22, 27, 30, 40	PBZ (1968) 7	7
28	PBZ 1 (1 + 6) 6, 9, 25	PBZ 1, 9	7, 10, 11, 18, 22, 25, 26	25
29	PBZ (1 + 6) 10 (13 + 1 + 6), 10, 18, 20, 25	PBZ 7, 10, 18, 22, 29	PBZ (1970) 6, 25, 27, 30, 61	22, 27
30	PBZ 10, 18, 22, 32	PBZ	18, 22, 25, 49	22
31	PBZ OPB 7, 11, 13, 18, 25, 60	OPB 18, 36, 60	6, 7, 25, 26, 28	7, 18, 22, 25, 28
32	PBZ OPB 7, 10, 22	OPB	7, 18, 25	7, 18, 25, 56
33	PBZ 6, 7, 10, 14	PBZ OPB 6	PBZ (1969) OPB (1966) 7, 9, 22, 59	7, 22, 59
34	PBZ (PBZ + 9) 18	PBZ	PBZ (1971) 1, 7, 10, 18, 22, 25	1
35	PBZ 1, 6, 7, 25, 28	PBZ 1, 9, 28, 29	PBZ (1971) 11, 22, 25, 47, 48, 58	11, 25, 57, 58
36	PBZ (PBZ + 1) 6, 7, 25	PBZ	PBZ (1961) 1, 6, 9, 25, 28	1
37	PBZ OPB 7, 18	PBZ 22	PBZ (1966) OPB (1969) 9, 16, 22, 25, 30	8, 22, 28
38	PBZ 1, 25	PBZ 1, 22	OPB (1968) 1, 6, 7, 9, 10, 12, 13, 20, 25, 26, 32	1, 22
39	PBZ 7, 18	PBZ	PBZ (1970) 6, 7, 13, 25	7, 18, 25
40	PBZ OPB 7	OPB	PBZ (1970) 1, 5, 7, 9, 14, 22, 39, 55	1, 22
41	PBZ 6, 16, 25	PBZ	7, 10, 18, 23, 25	7, 8, 25
42	OPB 1, 18, 41	OPB 1	7, 8, 22, 25, 60	7, 18, 22, 60
43	PBZ 6, 7, 25	PBZ 7	PBZ (1960) 1, 16, 25	1, 16
44	PBZ 7, 25, 28	PBZ 16, 27, 50	7, 10, 18, 25	18, 22

Year in brackets indicates when drug was last taken.

and unexposed controls. In order to look for any possible difference due to PBZ or OPB exposure, even at the expense of disregarding the original pairing of test and control subjects, a test of significance was applied to the 44 test patients against the 19 unexposed controls. Further comparison between the 69 patients exposed to PBZ or OPB (44 test and 25 controls) and the 19 controls never exposed was also made. No significant differences were found in any comparison (value for $P > 0.05$ to 1.0).

Comparison of the frequencies of Cu + Cs and R + D between male and female patients did not show any significant difference ($P > 0.10$ for both types of chromosomal aberration classes).

Discussion

The patients in this study are not comparable with a random sample from the general population. All have had rheumatoid arthritis for varying periods of time

Table IV Cytogenetic findings

Patient pair no.	Test patients					Control patients				
	T	B	Cu	Cs	(R + D)	T	B	Cu	Cs	(R + D)
<i>Female</i>										
1*	1	1	0	0	0	3	0	3	0	0
2*	0	0	0	0	0	4	2	2	0	0
3	1	0	1	0	0	5	3	2	0	0
4	2	1	0	1	0	3	1	2	0	1
5	3	3	0	1	0	6	3	3	0	0
6*	1	0	0	1	0	2	0	2	0	0
7	6	2	4	0	1	7	3	3	1	1
8	6	4	2	0	0	2	1	1	0	0
9*	7	4	2	1	0	3	2	1	0	0
10*	4	1	3	0	0	6	2	4	0	1
11*	2	0	2	0	0	4	2	2	0	0
12*	11	3	8	0	0	2	2	0	0	0
13	7	3	4	0	0	6	4	1	1	0
14	3	0	3	0	0	7	4	3	0	0
15*	5	2	3	0	0	5	3	2	0	0
16*	7	3	4	0	0	3	1	2	0	0
17	6	2	4	0	0	3	1	3	0	0
18*	1	0	1	0	0	3	3	0	0	0
19	1	0	1	0	0	5	2	3	0	0
20	2	2	0	0	0	5	3	2	0	1
21	6	5	1	0	0	5	1	4	0	0
22	3	0	3	0	1	8	6	3	0	1
23	2	2	0	0	0	5	3	2	0	1
24*	4	3	0	1	0	3	1	2	0	0
25	3	0	2	1	0	3	3	0	0	0
26*	1	1	0	0	0	8	6	1	1	1
27	11	5	4	2	1	4	2	2	0	1
28*	4	2	2	0	1	1	1	0	0	0
29	4	2	2	0	0	4	2	2	0	0
30*	5	3	2	0	0	10	5	4	1	0
31*	3	1	2	0	0	8	2	6	0	0
<i>Male</i>										
32*	3	2	1	0	0	2	1	0	1	0
33	2	0	1	1	0	5	2	2	1	1
34	2	2	0	0	0	5	3	2	0	0
35	8	3	2	3	1	10	6	3	1	0
36	3	3	0	0	0	7	5	2	0	0
37	2	1	1	0	1	5	2	2	1	0
38	1	1	0	0	0	7	3	4	1	1
39	6	2	3	1	0	4	2	1	1	0
40	2	1	1	0	0	3	2	1	0	1
41*	1	0	0	1	0	2	1	1	0	0
42*	6	3	3	0	2	2	1	1	0	0
43	6	4	2	0	0	5	3	0	2	0
44*	5	3	0	2	0	4	3	1	0	1

* Controls never exposed to PBZ or OPB.

T = total chromosomal damage (number of cells showing chromosomal damage); B = chromatid damage (break or gap); Cu = unstable chromosome arrangement; Cs = stable chromosome arrangement; R + D = rings and dicentric.

100 cells counted in all patients with the following exceptions. Test patients 18 (26 cells counted), 19 (39 cells counted), 20 (50 cells counted), 23 (63 cells counted).

Table V Percentage of cells showing chromosomal damage

Patients	%(Cu + Cs)	%(R + D)
44 Test	2.15 (90/4178)	0.19 (8/4178)
25 Controls (previously exposed)	2.48 (62/2500)	0.36 (9/2500)
19 Controls (never exposed)	1.95 (37/1900)	0.16 (3/1900)

Actual cell counts are shown in brackets.

and all have had a variety of drug therapy and diagnostic x-ray exposure. It would probably be false therefore to relate findings even in controls never exposed to PBZ or OPB with those in published reports where the general population have been sampled. Nevertheless, the level of chromosomal damage scored as Cu + Cs in the largest general population sample (Court Brown, Buckton, Jacobs, Tough, Keunssberg, and Knox, 1966), combining their results for short-term lymphocyte culture from both

Table VI *Table of analyses*

Row	Comparison	Test	No. of usable pairs	Smallest R value	z	χ^2	P
1	All 44 pairs (test v. controls) % of cells showing any type of chromosomal damage	W sig	41	315.5	1.00	—	>0.10
2	Cu + Cs	W sig	35	256	0.97	—	>0.10
3	R + D	W sig	13	32.5	0.96	—	>0.10
		Y	—	—	—	1.08	>0.10
4	19 pairs (test v. controls never exposed to PBZ or OPB) % of cells showing any type of chromosomal damage	W sig	18	77	—	—	>0.10
5	Cu + Cs	W sig	16	64.5	—	—	>0.10
6	R + D	F	—	—	—	—	1.00
7	44 test v. 19 controls never exposed to PBZ or OPB Cu + Cs	W sum	—	577.5	0.46	—	>0.10
8	R + D	F	—	—	—	—	1.00
9	69 (test and exposed controls) v. 19 non-exposed controls Cu + Cs	W sum	—	826	0.20	—	>0.10
10	R + D	F	—	—	—	—	0.75
11	Control patients only (25 exposed v. 19 non-exposed) Cu + Cs	W sum	—	350.5	1.82	—	>0.05
12	R + D	F	—	—	—	—	0.18

W sig = Wilcoxon's signed ranks test; W sum = Wilcoxon's sum of ranks test; Y = Yates's χ^2 test (d. of f. = 1); F = Fisher's exact test.

sexes, was 1.96% for ages from 15 to 75+ years, and 2.08% for ages from 35 to 74 years, indicating that values in the region of 2.0% are not exceptional. The age range of our patients was 32 to 74 years and the three Cu + Cs levels (ranging from 1.95% to 2.48%, see Table V) are not significantly different from one another nor from the levels given by Court Brown and others (1966).

Our findings therefore do not support those of Stevenson and others (1971) in that there is no significant increase in chromosomal damage in lymphocytes of patients exposed to PBZ or OPB. The difference between our results and those of Stevenson and others (1971) is not in the level of damage found in patients after PBZ or OPB therapy, but in the levels of the respective control series. Stevenson and others (1971) found a level of Cu + Cs in controls of only 0.48% whereas we found one of 1.95% in the patients never exposed to PBZ or OPB. We also differ in the levels of R + D found in both the test and control patients series. Our values of 0.16% for unexposed controls and values of 0.19% to 0.36% for exposed patients are between the corresponding values (0.06% for unexposed and 0.55% for exposed patients) by Stevenson and others (1971).

From the randomly selected general population (ages 15–75+ years) investigated by Court Brown and

others (1966) only 0.06% cells showed dicentric chromosomes (Court Brown, 1967). This value included some cells harvested from cultures where cells could have been in second or later division cycle. Allowing for the possible loss of some unstable chromosomal arrangements in late cultures, and including ring chromosomes it is still unlikely that the general population frequency of R + D would be >0.1%. This is supported by other investigations as reported in UNSCEAR (1969). All our R + D levels are therefore higher than those usually found in normal individuals. The values we found (from 0.16% to 0.36%), however, do not show any statistically significant differences between the test and control series of patients (Table VI).

If there is a real increase in chromosomal damage in our series of patients over that in the normal population, the available evidence does not indicate that PBZ and/or OPB are the cause. Table I shows two significant differences in functional grade and duration of rheumatoid arthritis; in both the test patients have a higher mean value than in the control series. Similarly, among the control patients the duration of disease was greater in those exposed to PBZ and/or OPB than in the unexposed, but there was no significant difference in chromosomal damage. Neither functional grade, nor duration of disease appear to

influence the chromosome results. All individuals (test and control) have been given other drug therapy and diagnostic x-rays, the most common drugs other than PBZ or OPB being sodium aurothiomalate and indomethacin. No association has been found in our series, however, between therapy with either of these drugs (at the time of the study or in the past) and chromosomal damage ($P > 0.10$).

Most fatalities from PBZ (and OPB) arise from marrow damage usually appearing as a sudden marrow hypoplasia. This is probably a threshold response in a population with variable sensitivity to

the drug. The sudden hypoplastic response may be due to a rapid decline in DNA synthesis. Investigations are proceeding on marrow specimens from a similar patient series.

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