TUMOURS OF THE URINARY BLADDER IN WORKMEN ENGAGED IN THE MANUFACTURE AND USE OF CERTAIN DYESTUFF INTERMEDIATES IN THE BRITISH CHEMICAL INDUSTRY*

PART II. FURTHER CONSIDERATION OF THE ROLE OF ANILINE AND OF THE MANUFACTURE OF AURAMINE AND MAGENTA (FUCHSINE) AS POSSIBLE CAUSATIVE AGENTS

BY

R. A. M. CASE and JOAN T. PEARSON

*From the Chester Beatty Research Institute, the Institute of Cancer Research,
University of London*

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In a previous communication (Part I) on occupational bladder tumours (Case, Hosker, McDonald, and Pearson, 1954), the primary object was to consider the statistical evidence which had been collected about the manufacture and use of the dyestuff intermediates aniline, benzidine, α -naphthylamine, and β -naphthylamine, and their relation to occupational tumours of the urinary bladder. It was concluded that aniline manufacture and use were probably not a cause of the disease, but that certain aniline workers who were employed in the manufacture of magenta (fuchsine) might be in some danger of developing occupational bladder tumours. At the same time it was suggested that the manufacture of auramine might also be a source of danger, and arrangements were made for a more detailed investigation of the occurrence of bladder tumours in workmen exposed to the manufacture of magenta, in which process aniline is necessarily used, and the manufacture of auramine, where aniline is not used.

This investigation, being part of the A.B.C.M. Papilloma Research Scheme (Association of British Chemical Manufacturers, 1953), was of necessity confined to the same firms as had hitherto been investigated and the data relate only to the manufacture of magenta and auramine. These substances differ from the other substances so far studied

in that they are finished dyes, and will be used by a different industry from that being investigated. Thus the conclusions to be drawn from the results presented below do not mean that the finished products magenta and auramine are necessarily causal agents; the data in this study do not enable this point to be elucidated. No investigation of the industry using these substances has been made.

This paper is intended to be complementary to Part I. The limitations of the statistical analysis discussed therein remain valid, the sources of information are the same, and any special terms previously defined are used with the same meaning.

Historical

It is perhaps a fruitless speculation to attempt to assess the true aetiological role of aniline in the cases attributed to this substance in the earlier literature, since frequently no clear distinction was made between aniline contacts and magenta contacts, and a review of the literature on impurities in industrial aniline used in earlier times (Walpole, Williams, and Roberts, 1952) reported the presence of α - and β -naphthylamine and 4-aminodiphenyl in the material. Subsequently 4-aminodiphenyl has been shown to produce bladder tumours in dogs following the oral administration of the material (Walpole and others, 1954). A list of cases attributed by various authors to aniline was given by Berenblum in 1932 and in a publication from the International Labour Office in 1921, and reviewed

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more recently by Hueper (1938). The following work relates only to industrial experience with aniline, magenta, or auramine between 1910 and 1952 in the firms investigated in the British chemical industry.

Data, Methods, and Results

Nominal rolls of men engaged in the manufacture of auramine and magenta were supplied by Group I* firms who had contributed nominal rolls to the original investigation. A careful cross-check was made to eliminate men who were recorded as also having had contact with benzidine, α - or β -naphthylamine. As before, men who had started work before 1910 with the firm submitting the nominal roll were rejected. As will be seen later, two men who subsequently developed bladder tumour were properly placed on the nominal roll since they started work with the firm after 1910, but the investigation of their history showed that they had previous experience elsewhere with auramine and magenta manufacture, though no contact with the naphthylamines or benzidine. They were therefore allowed to remain on the nominal roll, but the extra experience was added to the induction and exposure times. One case of a bladder tumour was encountered where the lapse of time from first starting work in the manufacture of auramine to developing a tumour was three months. By definition this man did not meet the minimum requirement of six months' employment in the industry to be included on the nominal roll, and, since some might

argue that it is highly unlikely that such a tumour was occupational, it has been thought advisable to omit the case from all the statistical procedures. This does not mean that the authors absolutely reject the possibility that the case was in fact occupational.

After the nominal rolls submitted had been scrutinized in this way, it was possible to divide them into the following exposure classes: (a) those men who had had contact with aniline but not with magenta, auramine, benzidine, α - or β -naphthylamine. The contact might have been in manufacture or use; (b) those men who had manufactured magenta, but who had not come into contact with auramine, benzidine, α - or β -naphthylamine; (c) those men who had manufactured auramine, but who had not manufactured magenta or come into contact with benzidine, α - or β -naphthylamine; (d) a small group of men who had manufactured both auramine and magenta, but who had not come into contact with benzidine, α - or β -naphthylamine.

The nominal roll for each exposure class was then set out as described in Part I and the expected number of death certificates at the mean rate for males in England and Wales computed by the comparative composite cohort analysis method described by Case (1953a, b) and employed in Part I. This calculation was made to include experience to the end of 1952, a later date than that used in Part I. In the new nominal rolls for magenta and auramine workers there was no class of men with incomplete data, although in the estimate for aniline workers

* Those firms taking part in the investigation which manufacture aniline, benzidine, α -naphthylamine or β -naphthylamine (see Part I).

TABLE 1

NO. OF DEATH CERTIFICATES EXPECTED IF NO SPECIAL RISK WERE OPERATING AND THE NO. OF CASES AND DEATH CERTIFICATES FOUND FOR VARIOUS EXPOSURE CLASSES

Rank	Class	Group*	Total No. of Cases Found	Cases on Nominal Roll			Cases on Nominal Roll where Death Certificate Mentions Bladder Tumour	Expected No. of Such Cases	No. of Men on Nominal Roll	Significance of Difference P	
				Total	Alive	Dead					
1	Aniline without benzidine, naphthylamine, magenta or auramine contact	I	3	3	2	1	1	0.52	812	None	>0.1
		II	0	0	0	0	0	0.30	394	None	>0.1
		III	0	0	0	0	0	0.01	17	None	>0.1
		All	3	3	2	1	1	0.83	1223	None	>0.1
2	Magenta contact	I	6	5	2	3	3	0.13	85	Very high	<0.005
3	Auramine contact	I	9	8	2	6	6	0.45	238	Very high	<0.005
4	Both magenta and auramine contact	I	1	1	1	0	0	0.02	9	None	>0.1
5	Either auramine or magenta contact (total of ranks 2, 3 and 4)	I	16	14	5	9	9	0.60	332	Very high	<0.005

*Group I, those firms which manufacture any one or more of the following substances: aniline, benzidine, α -naphthylamine, or β -naphthylamine; Group II, those firms which use but do not manufacture any of these substances, excluding those firms whose usage is confined to the purification of the substances for sale as fine chemicals; Group III, those firms which only purify any of these substances.

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the proportion derived from incomplete data remains as shown in Part I, Table 2.

The nominal rolls were also checked against available hospital records and the alphabetical list of death certificates for bladder tumour discussed in Part I and by Case (1953a, b). The number of verified cases found and the expectation of cases if no unusual risk was operating are shown in Table 1. The nominal rolls are not set out in full, but the total number of men on them is shown.

It is concluded from Table 1 that there is no evidence whatsoever to suggest that the manufacture or use of aniline, under the conditions pertaining in the British chemical industry between 1910 and 1952, has been a cause of occupational bladder tumour.

It is also concluded that, despite the small groups available for study, there is good statistical evidence to suggest that the manufacture of both auramine and magenta carries with it an occupational hazard of contracting bladder tumour. This does not mean that the finished products, auramine and magenta,

are the causal agents, and this analysis cannot help to elucidate this point.

The details of the cases found amongst the magenta and auramine cases are shown in Table 2.

In Part I it was shown that both the age at death and the age at onset were earlier in the occupational cases than in the general population and it was suggested that this could be a useful criterion of an environmental hazard. The present series of magenta and auramine cases is too small to be examined class by class but when amalgamated can be analysed.

The most precise method available for estimating the distribution of the ages at death that would be expected if it were similar to that in the general population is to use the partial sums of the composite cohort analysis calculation already made for Table 1 (see statistical appendix). This has accordingly been done, and a 2×2 contingency table drawn up to show the observed and expected deaths at ages of under 60 and 60 and over. This is shown in Table 3.

TABLE 2

DETAILS OF CASES OF BLADDER TUMOUR ONLY IN CONTACT WITH MAGENTA OR AURAMINE

Date of Onset	Age at Onset	Age at Death	Exposure Time in Years	Induction Time in Years	If on Nominal Roll	If Death Certificate Mentions Bladder Tumour	Class
1902	42	42	12	12	No	Yes	Magenta contact
1925	54	54	5	5	Yes	Yes	
1929	56	57	19*	28*	Yes	Yes	
1943	39	—	16	18	Yes	—	
1950	53	53	5	19	Yes	Yes	
1952	47	—	1	20	Yes	—	Both magenta and auramine contacts
1945	47	—	22	22	Yes	—	
1927	67	75	20*	20*	Yes	Yes	Auramine contact
1930	66	84	6	9	Yes	Yes	
1934	53	58	10	24	Yes	Yes	
1940	60	—	22	22	Yes	—	
1941	40	40	3	9	Yes	Yes	
1946	67	67	9	27	Yes	Yes	
1947	47	—	0.25†	0.25†	No	—	
1951	49	50	3	27	Yes	Yes	
1952	52	—	2	28	Yes	—	

*These two men were first employed by the member firm after 1910, and are thus properly included in the nominal roll. Both men had additional previous exposure to the relevant substance only and this additional time has been added in.

†This case is excluded from all statistical procedures.

TABLE 3

AGE AT DEATH AND AGE AT ONSET COMPARED WITH ESTIMATES MADE FROM SAMPLES OF THE GENERAL POPULATION

Age at Death	Under 60 Years	Over 60 Years	Total		Test of Significance
Nominal roll cases with bladder tumour death certificates only	6	3	9	Found	$\chi_c = 2.68$
	2.1	6.9	9	Expected*	$m = 2.1$ $p = 0.23$ $P = 0.007$
Age at onset	Under 50 Years	50-64 Years	Total		
	10	3	13	Found	$\chi_c = 3.95$
	3.3	9.7	13	Expected	$m = 3.3$ $p = 0.25$ $P < 0.005$

* The source of the line "expected" is discussed in the text.

Analysis by Fisher's exact test, using the χ_c technique (Fisher and Yates, 1948, Table VIII), shows the shift in the age at death towards the younger ages to be statistically significant.

The expected age at onset cannot be evaluated in this way, since no tables of morbidity data exist. The best means available to form the required estimate is to use the data relating to Birmingham hospitals used in Part I, limiting the upper age to 65 to avoid retirement bias, and to use all observed cases, whether derived from the nominal roll or not. A 2×2 contingency table on this basis also appears in Table 3, showing the observed and expected cases with an onset before the age of 50 and at 50 and over. (These lower figures are selected because of the

survival time from onset to death.) A χ_c analysis shows the lower age of onset to be statistically highly significant. It must therefore be concluded that the age at both onset and death in the occupational tumours of the bladder is lower than in the non-occupational cases.

The mean induction time of the 15 cases in the magenta and auramine classes shown in Table 2 (one of the 16 in the table is excluded) is 19.3 years with a standard deviation of 7.6 years. This does not differ significantly from the mean induction time (17.7, S.D. 7.2 years) found for the pooled data for benzidine, α - and β -naphthylamine cases discussed in Part I.

Summary

New nominal rolls of men exposed to the manufacture of magenta (fuchsine) and auramine have been prepared, and the previous aniline nominal roll has been revised by the removal of men exposed to the manufacture of magenta or auramine. It has therefore been possible to reconsider the evidence relating to the risks of contracting occupational tumour of the bladder in workmen engaged in these processes.

No statistical evidence has been found to suggest that the manufacture or use of aniline in the British chemical industry between 1910 and 1952 has caused occupational tumours of the bladder. This analysis is not necessarily relevant to the observations of other authors in other countries or in earlier times on cases attributed to aniline.

The manufacture of auramine or magenta in the British chemical industry between the same dates appears to have a definite occupational hazard of causing tumour of the urinary bladder attached to it, a statistically significant excess in the number of deaths certified as being due to bladder tumour being found, and the age of onset and death from the disease in the cases found amongst these manufactures being earlier than in the general population.

This statistical analysis is not capable of determining the causal factor in this risk attached to the manufacture of magenta and auramine, and it should not be assumed that the finished products are necessarily dangerous. No studies on the use of the finished products have, however, been made.

We should again like to thank those firms which undertook the laborious task of compiling the nominal rolls, and also Professor F. Grundy for certain criticisms that led to the use of the partial sum of the composite cohort analysis being used to estimate the expected age distribution.

REFERENCES

- Association of British Chemical Manufacturers (1953). *Papilloma of the Bladder in the Chemical Industry*. London.
 Berenblum, I. (1932). *Cancer Rev.*, 7, 337.
 Case, R. A. M. (1953a). *Brit. J. prev. soc. Med.*, 7, 14.
 — (1953b). *British Journal of Industrial Medicine*, 10, 114.
 —, Hosker, M. E., McDonald, D. B., and Pearson, J. T. (1954). *Ibid.*, 11, 75.
 Fisher, R. A., and Yates, F. (1948). *Statistical Tables for Biological, Agricultural and Medical Research*, Table VIII, 3rd ed. Oliver and Boyd, London.
 Hueper, W. C. (1938). *Arch. Path.*, Chicago, 25, 856.
 International Labour Office (1921). *Cancer of the Bladder among Workers in Aniline Factories*. Studies and Reports, Series F., No. 1. Geneva.
 Walpole, A. L., Williams, M. H. C., and Roberts, D. C. (1952). *British Journal of Industrial Medicine*, 9, 255.
 —, —, —, (1954). *Ibid.*, 11, 105.

STATISTICAL APPENDIX

A METHOD OF CALCULATING THE AGE-DISTRIBUTION OF DEATHS OCCURRING AT THE DISTRIBUTION EXPECTED FROM THE GENERAL POPULATION BY THE PARTIAL SUMS OF THE COMPOSITE COHORT ANALYSIS

In both Part I and Part II of this work the method of obtaining an estimate of the expected age-distribution of deaths from bladder tumour by a partial summation of the composite cohort analysis (Case, 1953b) sum is mentioned. It seems convenient to explain this procedure in more detail as an appendix, but reference to the tables given in "The Expected Frequency of Bladder Tumours in Works Populations" (Case, 1953b) is essential to an understanding of this exposition.

If we consider Table 2 of that paper we find the number of men of a defined age group Z set out in accordance with the time when they started work. They are then observed for a period of years defined as X until the date of "assessment", and an estimate of the total number of death certificates expected is found for that particular group of people from the product column ($n \times a \times b$)

divided by 10,000. If, instead, we subdivide the period X into a series of convenient periods, X_1, X_2, X_3 , etc., each of which now has a new mid-point, the original estimate $n \times a \times b \div 10,000$ can be regarded as the sum of the estimates for the period $X_1, X_2, X_3, \dots$ etc., but each such estimate will refer to a different age group, viz., $Z_1, Z_2, Z_3, \dots$ etc. This procedure is then employed throughout the whole calculation, and the contribution of each $Z_1, Z_2, Z_3, \dots$ etc., summated separately. From the grand total and these sub-totals, the proportionate distribution to each age group can be calculated. Computing may be minimized by considering the number of classes of Z that will be necessary; e.g., in Part I (Table 2a) the number of observed cases available allowed the use of four such classes, whereas in Part II (Table 3) only two could be used.