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CHROMOSOMAL INVESTIGATIONS IN CHEMICAL WORKERS.

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1. Chromosomal Investigations as a Plant Medical Precautionary Measure.

Since it has become possible to carry out chromosomal investigations relatively simply and routinely, chromosomal aberrations have been found by numerous investigators, which appeared after exposure to ionizing radiation (18), ingestion of various drugs, for example cytostatics (4), virus diseases (8,10,20) or the action of chemical substances and materials, for example, lead (15), benzene (3,4,17) or arsenic (12).

Even though the optimum technical reorganization of the workplace--through the practically completely automatic chemical reaction processes taking place predominantly in closed production systems--nearly excludes an exposure of our workers to toxic substances, there is a possibility of action by chemical materials in the case of plant disturbances which might arise. Therefore, in the large region of plant medical precautionary measures, the investigation of chromosome injuries in chemical plant workers was included.

We have started the investigation with chemical workers who deal with the substances which presumably produce chromosomal changes, or with those--at higher dosages--which are already known to do this (3,5,9,11,13,14,17,19). Being considered are chemicals in the alphabetical sequence

- ethyleneimine,
- benzene,
- lead,
- dimethyl sulfate.

Chromosomal investigations of chemical plant workers with
ethyleneimine,
benzene,
lead and dimethyl sulfate exposure.
Control group: boiler makers

Figure 1.

The control investigations were carried out on boiler-makers (Figure 1).

In addition, in vitro experiments were also carried out with the substances mentioned, in which the substance was added at a specific concentration to lymphocyte cultures of healthy, unexposed control subjects.

In the present paper, these experiments will only be mentioned and will be presented in detail in a later communication.

2. Investigational Procedure.

The chromosome analyses were carried out on lymphocyte cultures from venous blood according to the somewhat modified method of Moorhead. The growth of the cultures took place with addition of phytohemagglutinin and TC 199 of the firm DIFCO Detroit was used as nutrient medium. After 72 hours, the cultures were stopped with colchicine, after an additional incubation for 2 hours there followed a treatment with hypotonic solution and subsequently the fixation with glacial acetic acid-methanol. The air-dried preparations were dyed with 2% acetic acid orcein and embedded in Eukitt. The chromosomes of the lymphocyte nuclei in the stage of the metaphase can then be seen very well.

Figure 2 shows a normal chromosome metaphase of a lymphocyte. The chromosomes were arranged according to the Denver classification and a karyogram was set up of everyone investigated (Figure 3).

One hundred metaphases were analyzed and photographed for documentation of everyone investigated.

Figure 2.
Normal Metaphase of a Healthy Man.

Karyogramm
Boilermaker R.W.

Karyotype: 46 XY
Evaluation: Normal karyogram

Figure 3.
Normal Karyogram of a Healthy Man
(Denver scheme).

Chromosomal aberrations can occur either numerically--when the number of chromosomes deviates from the norm--or structurally--when, with a normal number of chromosomes, morphological changes are recognizable. However, even in healthy control subjects there is

Figure 4.
Metaphase of a Healthy Man with an Achromatic Lesion.

Karyogram
Boilermaker F.M.

Karotype: 46 XY
Evaluation: Achromatic lesion of a chromosome
17 of group E.

Figure 5.
Karyogram of a Spontaneous Chromosome Aberration.

certain percentage of so-called chromosome aberrations. Dealt with in these anomalies are chiefly structural ones, such as chromosome gaps or chromatin breaks; numerical chromosome disturbances, such as aneu-

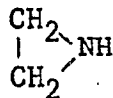
ploidies or hyperploidies, are rare. Data in the literature indicates that the frequency of spontaneous chromosome aberrations is located at 1-5% (1,6,7,16,17).

Figures 4 and 5 show a spontaneous chromosome aberration and a karyogram.

3. Investigational Results of the Chromosomal Analyses in the Individual Groups of Chemical Plant Workers.

3.1. Exposure to Ethyleneimine.

Chromosome changes by ethyleneimine,



a substance with a strongly alkylating action, have been reported in a few publications (13,14,19). Also, it is known to us that Dr. Kilian (Dow Badische, Freeport/Texas) has carried out chromosomal investigations for a number of years on workers who have handled ethyleneimine.

Due to the high toxicity of ethyleneimine, both through injuries to health in man and through very comprehensive animal tests, it appeared appropriate to investigate these chemical plant workers especially (Figure 6).

From the table, the long years of exposure of the workers is evident. The room air analyses carried out regularly, gave a value of less than 0.5 ppm of ethyleneimine.

The fact that chromosome changes, above those customary in the average population, were not observed in any of the subjects investigated, speaks well for the optimum technical rearrangement of the production unit.

3.2. Exposure to Benzene.

A number of publications are present relating to chromosome

No.	Name Date of birth	Joined BASF	Ethylene- imine manufac- ture	Concentration measured in workplace mac 0.5 ppm	Poisoning Inhal- Skin ation	% chromosomal aberration (Moorhead lymph- ocyte culture)
1	B.J. 8/26/33	1954	1962	measured regu- larly since	1967/ 68/69	-- 2%
2	C. J. 6/14/31	1960	1964	1968 with Dräger tube;	-- --	3%
3	G. H. 6/2/35	1959	1959	always less than 0.5 ppm	1962/ 63/66	1968 2%
4	H. E. 3/20/29	1954	1966		-- --	1%
5	H. W. 7/23/30	1948	1950		-- --	4%
6	H. O. 12/15/35	1957	1957		1964/ 65	-- 3%
7	R. F. 7/27/12	1927	1950		1955/ 63	-- 4%
8	S. J. 12/15/35	1968	1968		1970	-- 1%
9	Sch.K. 9/18/27	1968	1968		-- --	3%
10	Sch.G. 11/12/30	1954	1966		1965	2%

Figure 6.
Chromosome Investigations of Workers in the
Ethyleneimine $\begin{smallmatrix} \text{CH}_2 \\ \text{CH}_2 \end{smallmatrix} \text{NH}$ Manufacture.

changes by benzene (3,5,17). Thus, Haberlandt and Menthe in 1971 (3) reported considerable chromosome aberrations in workers chronically exposed to benzene. These investigated workers were apparently subjected to a strong action of benzene and, in part, also had manifest hematological damage.

In our workers investigated (Figure 7), who had also been working in such a plant for many years--in which no benzene content was found in recent room air analyses, after testing with the Dräger trace gas apparatus--no indications of increased chromosome aberrations were found, also, in the general examination and in the blood picture no deviations from the norm were found. From this it may also be concluded that the technical rearrangement of the workplace is sufficient.

No.	Name Date of birth	Joined BASF	Ethylene- imine manufac- ture	Concentration measured in workplace mac 0.5 ppm	Poisoning Inhal- Skin ation	% chromosomal aberration (Moorhead lymph- ocyte culture)
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2	C. J. 6/14/31	1960	1964	1968 with Dräger tube;	-- --	3%
3	G. H. 6/2/35	1959	1959	always less than 0.5 ppm	1962/ 63/66	1968 2%
4	H. E. 3/20/29	1954	1966		-- --	1%
5	H. W. 7/23/30	1948	1950		-- --	4%
6	H. O. 12/15/35	1957	1957		1964/ 65	-- 3%
7	R. F. 7/27/12	1927	1950		1955/ 63	-- 4%
8	S. J. 12/15/35	1968	1968		1970	-- 1%
9	Sch.K. 9/18/27	1968	1968		-- --	3%
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No.	Name Date of birth	Joined BASF	Benzene manufac- ture since	Concentration measured in workplace (mac 0)	Inhal- ation	Poisoning Skin	Gener- al find- ings	Blood pic- ture	Phenol in urine mg- liter of urine	% chromosomal aberration (Moorhead lymph- ocyte culture
1	D. W. 9/24/32	1958	1960	Manufacture in com- pletely closed system	--	--	no f.	no f.	55 mg	2%
2	D. K. 6/26/26	1959	1959	No benzene content in room air on measurement with Dräger trace gas apparatus	--	1962	gastr. no f.	no f.		1%
3	E. J. 4/11/22	1954	1959		--	--	no f.	no f.	38 mg	2%
4	F. F. 4/7/34	1959	1959		--	1970	gastr. no f.	no f.	65 mg	1%
5	K. O. 11/1/23	1938	1946		--	--	no f.	no f.	55 mg	1%
6	K. H. 3/2/36	1958	1958		1967	--	no f.	no f.	39 mg	4%
7	R. J. 8/15/14	1934	1934		--	--	no f.	no f.		3%
8	R. M. 12/19/33	1953	1953		--	1965	no f.	no f.	37 mg	1%
9	Sch. K. 1/10/21	1954	1954		--	--	no f.	no f.	31 mg	2%
10	Z. K. 9/23/27	1961	1961		--	--	cerv. syndr	no f.	49 mg	3%

Figure 7.
Chromosome Investigations of Workers in Benzene Manufacture.

3.3. Exposure to Lead.

Chromosome aberrations are known also on chronic exposure to increased lead. In 1970, Schwanitz, Lehnert and Gebhardt (15) found chromosome aberrations between 20 and 30% in lead workers. However, all workers examined displayed an increases level of lead in the blood and had an increased excretion of delta-aminolevulinic acid.

In our workers, we also carried out a hemoglobin determination (photometric), a determination of the basophilic pitted cells (Manson coloring, in the dark field) and the porphyrine determination (according to Hoschek) and examined the lead blood level (using the atom absorption analysis) and the urine for detection of the delta-aminolevulinic acid* (using thin-layer chromatography according to Knapp).

Haas, Mache, Schaller and Valentin (2) have recently reported the supervision of lead workers with delta-aminolevulinic acid dehydratase as a valuable biochemical parameter.

The values found are presented in Figure 8. Referred to especially is the subject B. K., born Oct. 16, 1936, who was not able to work for 3 weeks with colic-like stomach pains. Being dealt with was a subacute lead intoxication after working with homogeneous lead coating. Here, in an examination of 200 metaphases, 14% of chromosome aberrations were found, predominantly as gaps and chromatid breaks. Here, in two checks, the delta-aminolevulinic acid displayed definitely increased values of 148 and 168 mg, respectively. Of interest is that in this case, chromosome changes above the norm are found (Figures 9 and 10).

*At this place we wish to thank Prof. Klingmüller of the medical clinic of the Heidelberg-Mannheim University, who carried out the investigations in a cooperative manner.

No.	Name Date of birth	Joined BASF	Lead exposure since	Concentration in workplace (mac = 0.2 mg/m ³ ; 200 μg/m ³)	General findings	HG photo- metrically	Basophilic cells Manson dark field 2%	Blood porphyrine Hoschek	Blood Pb atom. absorption	δ-aminolevulinic acid mg/kg Knapp thin-layer chromatography	Creatinine	% chromosome aberrations (Moorhead lymphocyte culture)
1	B. K. 10/16/36	1956	1956	Measured at 16 places	no f.	99	100/2f	-	0.6 ppm	148 mg*	0.74	14%**
2	E. A. 12/18/21	1957	1957	9-15 hours	no f.	110	100/1f	-	0.7 ppm	--	1.06	4%
3	F. H. 12/30/29	1954	1954	average concentration in workplace was 26 μg/m ³	no f.	104	--	+		--	0.405	5%
4	K. K. 8/1/23	1948	1948		no f.	100	100/1f	+	0.8 ppm	traces	1.990	3%
5	R. F. 1/10/42	1956	1957		no f.	106	100/1f	-	0.9 ppm	--	1.800	4%
6	R. J. 4/22/22	1951	1951		gastr.	101	--	+	0.6 ppm	--	1.190	4%
7	Sch. K. 5/22/40	1954	1954		no f.	105	--	-	ppm	--	0.650	4%
8	Sch. L. 6/9/31	1951	1951		no f.	103	--	-	0.6 ppm	--	1.010	5%
9	W. K. 1/10/28	1945	1945		no f.	113	100/18f ^{6g}	++	1 ppm	traces	1.060	3%
10	W. H. 12/5/37	1952	1952		no f.	99	100/1f	-		---	1.56	3%

*168 mg six weeks later

**200 metaphases were counted

Figure 8.
Chromosome Investigations of Lead Solderers.

Figure 9.
Metaphase of the Lead Solderer B. K.
with Chromosome Aberrations.

Karyogram
Lead solderer B. K.

Karyotype: 46.XY
Evaluation: Achromatic lesion of a chromosome
9 group C.

Figure 10.
Karyogram with Chromosome Aberration in Lead Solderer B. K.

3.4. Exposure to Dimethyl Sulfate.

Also in the case of dimethyl sulfate, which is strongly alkylating and is claimed to be a carcinogen in the VIIth communication of the Commission for Testing Harmful Industrial Materials/DFG in animal tests, we could find no chromosomal changes in our workers

No.	Name Date of birth	Joined BASF	Dimethyl sulfate manuf. since	Concentration measured in workplace (mac 0.01 vol ppm)	Poisoning Inhal- Skin ation	% chromosomal aberration (Moorhead lymph- ocyte culture)
1	B. K. 2/12/30	1951	1951	DMS processed in closed	-- --	1%
2	G. R. 12/28/30	1960	1960	system. No DMS could	-- --	1%
3	L. G. 10/12/22	1950	1950	be detected in repeated	1958 --	3%
4	M. W. 11/28/40	1963	1963	measurements with the	-- --	2%
5	Sch. K 8/29/31	1960	1960	Dräger trace gas appara-	-- --	4%
6	Sch. W 5/4/43	1958	1958	tus	-- --	3%
7	Sch. W 1/30/32	1953	1953		-- --	4%
8	T. G. 3/29/36	1959	1959		-- 1961 1970	2%
9	W. H. 1/22/34	1953	1953		-- --	3%

Figure 11.

Chromosome Investigations of Workers in Dimethyl Sulfate Manufacture.

(Figure 11).

Dimethyl sulfate is also processed in a completely closed system. The room air analyses gave no indication of dimethyl sulfate.

3.5. Chromosome Investigations of Workers from the Boilermakers (Figure 12).

In this group there is no exposure to chemical substances. Also, no chromosome aberrations above the norm were found in the karyograms.

4. In Vitro Experiments.

To supplement the investigations presented, we have started in vitro experiments in which the lymphocyte cultures of healthy normal persons were treated 24 hours before the end of the culture with the substances ethyleneimine, benzene, lead (lead acetate) and dimethyl sulfate in various concentrations. These results, which indicate concentration-dependent chromosome aberrations, will be reported later.

No.	Name Date of birth	Joined BASF	Boiler- maker since	% chromosomal aberration (Moorhead lymphocyte culture)	Remarks
1	B. L. 12/29/30	1945	1946	2%	Exposed to noise
2	F. J. 2/8/40	1955	1956	3%	
3	F. W. 4/18/36	1964	1964	3%	
4	G. W. 11/27/35	1950	1951	1%	
5	G. O. 1/25/38	1952	1953	2%	
6	L. H. 3/6/39	1953	1954	2%	
7	M. W. 8/18/32	1947	1948	1%	
8	R. G. 8/17/38	1952	1953	2%	
9	R. W. 11/30/41	1955	1956	2%	
10	U. E. 8/10/31	1946	1947	1%	

Figure 12.
Chromosome Investigations of Workers from the Boilermakers.

5. Discussion of Our Results and Summary.

The observations of chromosomal changes through chemical materials and substances, reported in the domestic and foreign literature, are the result of chronic and increased exposure. In the workers investigated an increased exposure to chemical substances can be excluded and, correspondingly, no increased chromosome aberrations were found. Only in the case of one lead solderer, whose clinical symptoms indicated a subacute lead poisoning, was the chromosome aberration, at 14%, above the norm.

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