SOME EARLY EFFECTS OF EXPOSURE TO TRINITROTOLUENE *

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It was felt that if we could determine the early effects of exposure to TNT, the later manifestations of severe poisoning, which occur in only a small percentage of people, might be better understood. In order to overcome the difficulties inherent in a large scale survey of factory workers, we decided to use volunteers who would be willing to work for a period in a filling factory and co-operate in a series of tests in Oxford before and immediately after the period of exposure. In this way we could try out an extensive series of tests and select those which would be most useful and practicable in subsequent investigations of the ordinary factory worker. Permission was obtained from the Ministry of Supply, and an appeal was made to the undergraduates of Oxford and several other Universities to spend part of the summer vacation doing their work. The number of volunteers easily exceeded the number that could be dealt with in the laboratory or conveniently handled at the factory. In the end 62 students were chosen. Physical examination and laboratory tests showed that all of them were healthy.† They went to the same Royal Ordnance Factory and were housed in one of the government hostels for factory workers. They had no special privileges and were treated as ordinary workers, receiving the same rates of pay and obeying the same rules and regulations. They were asked to stay a minimum period of four weeks and many stayed longer.

Description of Factory Work and Grouping of Students

Contact work for 42 of the students was done in the filling shops. In addition ten male students

spent part of their time in the melt houses. An analysis of the concentrations of air-borne TNT in the shops where the students were working was made for us by C. G. Warner‡ and the figures obtained are given in Table 1. If the students were taken off contact for any reason, usually because of dermatitis, they worked on trucking, fusing trench mortars, or in the stores. Fig. 1 shows the length of time spent by each individual in the different shops. There were two day shifts, each of which lasted 8 hours, with a canteen interval of half an hour in the middle, and one night shift of 9½ hours, with two shorter rest periods. There was a week-end break which varied from 18 to 36 hours according to the shift. The students wore the regulation overalls and caps, worked with bare hands and did not wear masks. In the statistical analysis of the data it was found desirable to subdivide the total population of students into three groups, which were homogeneous as regards sex and occupation. Group I (44 females) and Group II (8 males) worked in the filling shops, and the average exposure in both groups was 33 days. Group III (10 males) averaged 18 days in the filling shops and 15 days in the melt houses.

TABLE 1
CONCENTRATION OF AIR-BORNE TNT IN
FILLING SHOPS AND MELT HOUSES

Contact work	Av. conc. of TNT in mg. per cu. metre	
	Day	Night
Filling shops	0.3	0.6
Melt houses —		
Floor	0.3	0.3
Platform	1.2	1.3

The average age in all three groups was twenty years. The results for the three groups are given separately in the tables which follow.

* Report of an investigation carried out during the summer of 1942 at a Royal Ordnance Factory, under the auspices of the Medical Research Council.

† One of the female students had had catarrhal jaundice 3 years before. There were no signs of hepatic impairment at the initial examination and she experienced no ill effects from working at the factory except a slight anaemia, the haemoglobin falling from 89 to 80 per cent.

‡ Method described at the Medical Research Council Conference on TNT., 1942.

FIG. 1.—Duration of exposure to TNT and type of work done by 62 students at a Royal Ordnance Factory.

Before going to the factory, the volunteers spent two days in Oxford, where everyone was given a full clinical examination which included records of blood pressure, weight and urine analysis. On the afternoon of the first day they had a 4-hourly hippuric acid test. The following morning the students returned fasting to the laboratory and 20 c.c. of venous blood was collected in an oxalate tube. Samples from this single collection were used for the following tests:

Red blood cell count
Haemoglobin
Haematocrit
Blood urea
Plasma bilirubin
" cholesterol
" proteins
" phosphatase

A sample of capillary blood was collected for white cell count and reticulocytes. Immediately afterwards the student was given 100 gm. of sucrose (cane sugar) in 200 c.c. water and samples of venous blood were collected at the end of one, one and a half and two and a half hours, for determination of blood laevulose. On the afternoon of the same day most of the students visited the Oxford Nutrition Survey where their dark adaptation was tested and their eyes examined with the slit lamp, and a further sample of blood was collected for the following examination:

Vitamin C in plasma and white cells
Coccarboxylase
Riboflavine
Vitamin A and carotene

While the students were at the factory reticulocyte counts were taken every third or fourth day and the

TABLE 2

SUMMARY OF THE CLINICAL FEATURES. TNT SICKNESS INCLUDES VOMITING, ABDOMINAL PAIN, NAUSEA AND DIARRHOEA. MISCELLANEOUS SYMPTOMS INCLUDE NASAL AND THROAT IRRITATION, FATIGUE, MENSTRUAL DISTURBANCES AND OEDEMA

Students			Exposure						Symptoms										Weight		
Group	Number	Sex	Operation	Days			Rash			TNT sickness			Cyanosis			Hunger	Before	After			
				Average	Maximum	Minimum	Generalized	Hands only		Severe	Moderate	Slight	Severe	Moderate	Slight				Severe	Transient	Miscellaneous
								Severe	Moderate												
I	44	F	Filling ..	33	56	9	3	3	5	14	2	10	12	0	0	16	40	lb. 143	lb. 140		
II	8	M	Filling ..	33	54	20	0	1	0	4	0	0	4	0	0	3	8	140	135		
III	10	M	Filling ..	18	31	8	0	0	0	0	0	2	5	1	4	10	9	142	142		
			Melting ..	15	30	3															

urine was examined at frequent intervals for urobilin. A selected number collected 24-hourly urines for estimation of porphyrins, and samples were also examined for indican, and the Webster and Kennedy-Ingham reactions. These tests were repeated after leaving the factory. On two occasions research workers paid a visit to the factory to collect a batch of blood samples at the end of a shift for methaemoglobin estimations.

On return to Oxford the tests and clinical examinations were repeated. A careful history was taken, recording the exact number of days on contact, the work done, and the development and duration of symptoms. During the experiment it became apparent that haematological changes were occurring and the last three volunteers agreed to have a sternal puncture before and after exposure. This investigation was also made on two other individuals at their final examination, and was repeated three months later. Finally, a few individuals who had experienced abdominal pain were asked if they would submit to a gastroscopy, and four volunteers were examined in this way (three women and one man).

The following technical methods were used:

BLOOD

Haemoglobin

- (1) Carboxyhaemoglobin method of Haldane (British Standards Institution, No. 1079, 1942).
- (2) Alkaline haematin, colorimetric (Clegg and King, 1942).
- (3) Pyridine haemochromogen, colorimetric (Rimington, 1942).
- (4) Total iron, dry ashing and titanous chloride titration (Klump, 1934).

Reticulocytes

Supravital cresyl blue (Kracke, 1941).

Urea

Urease and nesslerisation (Archer and Robb, 1925).

Laevulose

Laevulose was determined in the blood before and after the ingestion of 100 gm. of sucrose by mouth (Higgins, 1942). The method of estimation is colorimetric (Patterson, 1935). The *laevulose index* was obtained by subtracting the fasting value of the blood laevulose in mg. per 100 c.c. from the values at 45, 90, 120 and 150 minutes after giving the sucrose, and adding together the figures so obtained (cf. Galactose Index, Barnes and King, 1943).

Methaemoglobin

Spectro-photometrical (Vigness, Watson and Spink, 1940).

PLASMA

Proteins

Micro-kjeldahl (Howe, 1921).

Bilirubin

Total bilirubin, modified Van den Bergh, colorimetric (Thanhauser and Anderson, 1921).

Direct and indirect bilirubin, photometric (Malloy and Evelyn, 1937).

Phosphatase

Colorimetric (King and Armstrong, 1934).

URINE

Hippuric acid test (Probstein and Londe, 1940).

Kennedy-Ingham test. Colour reaction (Kennedy and Ingham, 1942).

Webster test. Colour reaction (Webster, 1921).

Coproporphyrin. Fluorimetric and U.V. absorption (Jope, 1942).

DIRECT INDICAN REACTION. Among tests for the presence of abnormal substances in the urine was included the reaction for indoxyl potassium sulphate (indican). After the period of exposure a certain number of urines gave a direct indican reaction; that is, the addition of concentrated hydrochloric acid without the oxidizing agents usually used in the tests for indican, caused the formation of indican. This reaction was carried out in the following manner: 2 ml. of chloroform were placed in a graduated centrifuge tube, 5 ml. of urine were added followed by 5 ml. of concentrated hydrochloric acid. The tube was corked and shaken vigorously to ensure extraction of indigo by chloroform. If a blue colour developed in the CHCl_3 within 5 minutes, the test was considered positive. With most of the urines which gave positive reactions, the formation of indigo occurred within 1 to 2 minutes of addition of the acid. Often urines gave a positive reaction on standing for a greater length of time and those urines which gave an immediate positive reaction gave more intense reactions on standing. It is advisable to ignore these later reactions, since the concentrated hydrochloric acid may produce slowly oxidizing substances from the urinary constituents. An explanation of this immediate indican reaction is that nitro compounds, simultaneously excreted with indoxyl potas-

sium sulphate, act as oxidizing agents in the same way as the hypochlorites in the usual indican tests. A similar explanation has been offered for the direct indican test given by pellagrin's urines. (Meiklejohn and Kark, 1939.)

The Findings

The symptoms and clinical effects experienced by the students from work in contact with TNT were identical, not only in nature but also in frequency, with those recorded by Voegtlin in 1922 and by Noro in Finland in 1941. All the students were re-examined six months later and none showed any ill effects from the experiment.

GASTROSCOPY. Four of the students who had experienced gastro-intestinal disturbances were examined with the gastroscope on their return from the factory. Symptoms in two of them had appeared early. In both there was epigastric tenderness and deep pressure in the right hypochondrium had brought on a recurrence of the pain. The other two had had no symptoms until after five weeks' exposure, but pain and nausea had been present for at least two weeks. Abdominal examination in these two was essentially negative. The gastroscopic findings were similar in all four cases though they varied somewhat in severity. In each case there was an excessive amount of thick, ropy mucus which was adherent to the gastric mucosa. The latter was slightly paler than normal and had an a slightly smooth appearance. These changes were more marked in one case with severe symptoms than in the other three, and in this individual the gastric surface showed numerous high lights suggestive of oedema and excessive mucus. The resting juice was examined in each case and free acid was present. The changes observed are those commonly seen in mild cases of so-called catarrhal gastritis.

URINE TESTS. The urine of all the students was tested at the initial and final examinations in Oxford, and morning specimens were tested once a week while at the factory. Thirty-one students collected 24-hourly specimens before, during and immediately after exposure. The latter were used for estimation of coproporphyrin excretion and in addition certain special tests were carried out—Webster reaction, Kennedy-Ingham and direct indican reaction. These tests were repeated on all the urines at the final examinations. The routine examination included tests for albumin, sugar, acetone and urobilin.

At the initial examination a trace of albumin was found in two cases and acetone in one. No one had either sugar or urobilin. The urine tests carried out at the factory and on returning to Oxford gave a positive urobilin test in twenty-six students. Often it was only present on one occasion but in several it was found in two or more instances. Usually the test was a slight positive, but in seven cases it was strongly positive. Only one person (in Group III) had bile in the urine. A trace of albumin was found on one occasion only and acetone was present once in three individuals.

The coproporphyrin estimations gave figures within the accepted range of normal in every instance. The Webster test was positive in twenty-nine individuals, the Kennedy-Ingham in forty-two and the direct indican in eighteen. The Webster test has for long been accepted as an indication of absorption of TNT and is of no special pathological significance, though observers have commented on the fact that it becomes negative in cases of toxic jaundice. In this investigation, where the test was often repeated as many as four or five times in the same individual, it was frequently positive on one occasion and negative on another. The same variability was noticed in the Kennedy-Ingham reaction. The significance of the latter is not yet clear, but the original claim that a positive colour reaction indicated an increased excretion of coproporphyrin in the urine is not borne out by the present investigation as there was no correlation between it and the direct coproporphyrin estimations. There was no difference in the number of positive Webster and Kennedy-Ingham tests in the three groups, and no correlation with the clinical findings.

Indican (potassium indoxyl sulphate) is an important ethereal sulphate derived from the products of intestinal putrefaction and a positive test for indican in the urine is usually taken to indicate some intestinal disorder or an excessive ingestion of meat in the diet. In the present series fifteen of the eighteen individuals with a positive direct reaction had symptoms of TNT sickness. It should be noted, however, that the urine from normal individuals may occasionally give this reaction.

HAEMATOLOGY. The haemoglobin was checked by four independent observers, each of whom used a different method: (1) Total iron. (2) Haldane (standardized by N.P.L.). (3) Alkaline haematin. (4) Pyridine haemochromogen. For conversion into percentage values, 15.0 g. haemoglobin was made equivalent to 100 per cent. The values recorded in Table 3 are the average of all four methods in each case. There was a fall in haemoglobin, red cells and haematocrit reading in all three groups of students. The average percentage fall was slightly greater for the haemoglobin than for the erythrocytes but the alteration in colour index was negligible. The changes were greatest in Group III, especially in the haemoglobin values. In each series there was considerable variation in the individual results. In Group I the maximum fall in haemoglobin was 28 per cent., whereas seven cases showed a change of less than 4 per cent. and one had a rise of 8.5 per cent. In Group II the greatest individual drop was 23 per cent.; one case showed no change and one gave an increase of 10.5 per cent. on his original figures. In Group III every case gave a lower value after exposure and the drop ranged from 10 to 21 per cent. in every case except one where the fall was only 4 per cent. Similar variations were found in the red cell counts and the haematocrit percentage. Over 80 per cent. of all the students were adversely affected.

TABLE 3
BLOOD STATE BEFORE AND AFTER EXPOSURE TO TNT

In this and the other tables the mean values and the standard error of the mean are shown for each of the three groups of students. Where the figure after exposure is significantly different (i.e. the probability of error less than 1 in 100) it is in *italics*.

Investigations	Group I (44 females)			Group II (10 males)			Group III (8 males)		
	Before exposure	After exposure	Individual percentage difference	Before exposure	After exposure	Individual percentage difference	Before exposure	After exposure	Individual percentage difference
Haemoglobin, per cent. . .	94±1.1	84±1.0	-9.7±1.06	105±3.3	95±2.15	-9.5±3.4	106±1.55	91±0.88	-14.2±1.5
Erythrocytes, ×10 ⁶ per c.mm. . .	4.9±0.07	4.6±0.07	-6.2±1.58	5.4±1.43	4.9±1.43	-9.2±3.4	5.5±0.1	4.9±0.1	-10.5±1.4
Haematocrit, per cent. . .	41±0.59	39±0.48	-4.9±1.15	47±1.28	43±1.36	-8.5±3.1	48±1.17	43±1.13	-10.0±2.07
Reticulocytes, ×10 ⁴ per c.mm. . .	3.8±0.78	9.3±2.17	+140±1.67	3.6±0.75	12.6±2.6	+250±0.7	4.3±0.83	27.1±5.5	+530±1.47
Leucocytes, ×10 ³ per c.mm. . .	7.4±0.33	5.8±0.41	-22±3.96	6.2±0.64	6.1±1.1	-2±18.3	6.8±0.66	8.0±0.74	+18±13.1

Accompanying these changes there was an increase in the number of reticulocytes in the circulation. Reticulocyte counts were made at the beginning and the end of the experiment, and also every two or three days at the factory. For statistical purposes absolute values have been used rather than the more usual percentages. The average initial figure for all three groups was 40,000 per c.mm. and the normal range may be taken as 20,000-60,000 per c.mm. The increase was much greater in Group III than in either Group I or II, and in this group the average final value was over seven times the original. In Groups I and II the increase was approximately three times the mean initial value. Again the individual variation was considerable, and in Group I seven cases had a final count of less than 70,000 per c.mm. In Group III half the cases had counts of over 200,000 per c.mm. with a maximum of 530,000 per c.mm., and no one had a count of less than 70,000 per c.mm. In Groups I and II the maximum values were 290,000 per c.mm. and 260,000 per c.mm. respectively.

The final reticulocyte values recorded in Table 3 were those obtained on return to Oxford, 24-48 hours after leaving the factory. In almost every case this value was higher than any obtained during the period of exposure. The complete set of values obtained in one case (No. 47, Group III) throughout the period of exposure and for the four weeks following is given in Fig. 2. The last reticulocyte counts at the factory are compared with the values on return to Oxford in Table 4. The counts

TABLE 4
LAST RETICULOCYTE COUNT AT FACTORY COMPARED WITH VALUE ON RETURN TO OXFORD APPROXIMATELY 48 HOURS LATER

Group	Reticulocytes Average number per c.mm. blood (×10 ⁴)		
	Before exposure	Last count at factory	Two to four days after cessation of contact
I	3.8	7.5	9.3
II	3.6	8.1	12.6
III	4.3	15.0	27.1

at the factory in Groups I and II were only just outside the normal range.

The findings in the five sternal punctures are summarized in Table 5. One was a man from Group II and four were men from Group III. In all the cases from Group III the marrow, after exposure, was very cellular and showed a marked erythroblastic reaction. The nucleated red cells frequently occurred in nests and mitotic figures were very apparent. The number of erythroblasts showed an average increase of three times the resting value. The appearance of the marrow before and after exposure was essentially unchanged in the only case from Group II that was examined. This individual had shown no evidence of destruction of the circulating red cells.

METHAEMOGLOBIN. Blood from seventeen students was examined for methaemoglobin before departure, and the pigment was uniformly absent. Nine of these were re-examined at the factory, the blood being drawn immediately after the end of a shift. Three from Group I were all negative, two from Group II gave values of 0 and 3 per cent. respectively, while four from Group III all gave positive results ranging from 3 to 6 per cent. Five others were examined on their return to Oxford. The interval between collecting the blood and leaving the factory in these cases varied from thirty-six to sixty hours. The results in four of these (three from Group I and one from Group III) were negative. The remaining one was a man from Group III who had developed severe anilism.

FIG 2.—Total reticulocyte count during and after heavy exposure to TNT, showing crisis after removal from contact.

TABLE 5
RESULTS OF STERNAL PUNCTURE IN FIVE STUDENTS EXPOSED TO TNT

Sex	Group	Per cent. erythroblasts in sternal marrow			Haemoglobin, per cent.		Reticulocytes, $\times 10^4$ per c.mm.	
		Before exposure	Immediately after exposure	3 months later	Initial	Final	Initial	Final
M	III	—	46	14	115	94	4.6	50.0
M	III	10	31	—	100	96	7.7	38.0
M	III	—	33	20	99	89	6.1	52.8
M	III	16	35	—	116	93	3.4	44.1
M	II	23	28	—	86	92	6.0	5.3

TABLE 6
RESULTS OF LIVER FUNCTION TESTS (Cf. legend to Table 3)

Investigation	Group I			Group II			Group III		
	Before exposure	After exposure	Individual percentage difference	Before exposure	After exposure	Individual percentage difference	Before exposure	After exposure	Individual percentage difference
Total serum bilirubin, mg. per 100 ml. ..	0.38 $\pm$ 0.03	0.51 $\pm$ 0.06	+ 36 $\pm$ 12.1	0.75 $\pm$ 0.24	1.51 $\pm$ 0.51	+ 134 $\pm$ 67.5	0.45 $\pm$ 0.009	1.19 $\pm$ 0.32	+ 179 $\pm$ 90.0
Indirect bilirubin, mg. per 100 ml. ..	0.2 $\pm$ 0.003	0.3 $\pm$ 0.02	$\pm$ 85 $\pm$ 50.9	1.0 $\pm$ 0.72	1.4 $\pm$ 1.3	+ 40 $\pm$ 50.0	0.2 $\pm$ 0.07	1.35 $\pm$ 0.96	+ 410 $\pm$ 311.0
Direct bilirubin, mg. per 100 ml. ..	0.3 $\pm$ 0.003	0.3 $\pm$ 0.02	0 $\pm$ 50.9	0.5 $\pm$ 0.72	0.6 $\pm$ 1.3	+ 20 $\pm$ 50.0	0.2 $\pm$ 0.07	0.4 $\pm$ 0.96	+ 100 $\pm$ 311.0
Plasma phosphatase, units ..	7.3 $\pm$ 0.25	8 $\pm$ 0.45	+ 9 $\pm$ 1.69	9.8 $\pm$ 1.03	11.9 $\pm$ 2.9	$\pm$ 21 $\pm$ 33.5	8.3 $\pm$ 0.76	10.1 $\pm$ 1.0	+ 21 $\pm$ 13.6
Hippuric acid test, per cent excretion in 4 hours ..	88 $\pm$ 1.04	87 $\pm$ 1.21	- 1 $\pm$ 1.16	93 $\pm$ 0.68	87 $\pm$ 2.6	- 6 $\pm$ 3.4	93 $\pm$ 1.37	91 $\pm$ 1.5	- 2 $\pm$ 2.46
Laevulose index ..	21 $\pm$ 2.03	23 $\pm$ 1.74	+ 9.5 $\pm$ 4.2	22 $\pm$ 2.7	20 $\pm$ 1.29	- 9 $\pm$ 7.8	21 $\pm$ 1.42	20 $\pm$ 1.08	- 5 $\pm$ 8.4

TABLE 7
PLASMA PROTEINS BEFORE AND AFTER EXPOSURE TO TNT (Cf. legend to Table 3)

Investigation	Group I			Group II			Group III		
	Before exposure	After exposure	Individual percentage difference	Before exposure	After exposure	Individual percentage difference	Before exposure	After exposure	Individual percentage difference
Total plasma protein, gm. per 100 ml. ..	7.0 $\pm$ 0.05	6.7 $\pm$ 0.05	- 4.3 $\pm$ 0.8	7.4 $\pm$ 0.55	6.9 $\pm$ 0.15	- 6.7 $\pm$ 1.4	7.3 $\pm$ 0.16	6.7 $\pm$ 0.08	- 8.2 $\pm$ 1.4
Plasma albumin, gm. per 100 ml. ..	4.7 $\pm$ 0.04	4.5 $\pm$ 0.05	- 4.2 $\pm$ 1.08	4.9 $\pm$ 0.12	4.5 $\pm$ 0.11	- 8.1 $\pm$ 2.5	4.9 $\pm$ 0.08	4.5 $\pm$ 0.05	- 8.1 $\pm$ 1.76
Plasma globulin, gm. per 100 ml. ..	2.1 $\pm$ 0.05	2.0 $\pm$ 0.05	- 4.8 $\pm$ 2.84	2.2 $\pm$ 0.13	1.9 $\pm$ 0.12	- 13.6 $\pm$ 5.1	2.2 $\pm$ 0.06	1.9 $\pm$ 0.001	- 13.6 $\pm$ 7.8
Plasma fibrinogen, mgm. per 100 ml. ..	276 $\pm$ 8.1	266 $\pm$ 8.1	- 3.6 $\pm$ 2.8	308 $\pm$ 28.1	226 $\pm$ 16.0	- 21.6 $\pm$ 9.7	251 $\pm$ 10.3	253 $\pm$ 8.5	0.8 $\pm$ 5.4

Unfortunately five days had elapsed between his being taken off contact and his return to Oxford, nevertheless he still had 8 per cent. total non-oxygen carrying pigment in the blood and the "sulphaemoglobin" band (6210) was just visible in the spectrum.

PLASMA BILIRUBIN. Group I showed a slight but significant rise in the average level of the total serum bilirubin after exposure, i.e. 0.38-0.51 mg. per 100 c.c. Four individuals showed a rise varying from 0.5 to 1.9 mg. per 100 c.c. In Groups II and III the total bilirubin values after exposure were even higher, and the average final values were over 1.0 mg. per cent. If the figures from these two groups are analysed separately the individual variation is such that a significant difference cannot be established, but taking the two groups together

the difference is significantly different from zero. This suggests that it is reasonable to suppose that there was a systematic rise in bilirubin in all the three groups.

In one man in Group II the total plasma bilirubin was 2.4 mg. per cent. before exposure and 3.8 per cent. after. The other tests of liver function were normal and he felt no subjective ill effects. His haemoglobin fell from 99 to 89 per cent.

The man in Group III who developed anilism had an initial total bilirubin value of 0.6 mg. per cent. and a final value of 5.0 mg. per cent. It is noteworthy that his maternal grandfather and uncle had both died of cirrhosis of the liver. The man himself was liable to fat dyspepsia but otherwise fit.

In about half the cases (24 females, 6 males) the

amount of bilirubin that gave the direct reaction was estimated separately. The results showed that the rise in total bilirubin was largely accounted for by the rise in the indirect bilirubin, there being little or no change in the value for the direct. For instance, in the case from Group III with a final value of 5.0 mg. total bilirubin, 4.2 mg. gave the indirect reaction. In view of the evidence of destruction of circulating red cells, the rise in the plasma bilirubin was more likely to be due to haemolysis than to a toxic effect on the liver, nevertheless the fact that the liver was unable to maintain a normal plasma level while excreting the increased amount of pigment suggests some impairment of function. (Watson, 1937.)

PLASMA PHOSPHATASE. HIPURIC ACID EXCRETION. LAEVULOSE TOLERANCE. In these tests there was only a slight difference in the average values before and after exposure. In five cases there was a fall of more than 15 per cent. in the amount of hippuric acid excreted in four hours, and in four of the other cases a rise above 15 units in the plasma phosphatase. In no single case was the rise in laevulose index greater than the corresponding fall in other instances, suggesting that the changes observed did not fall outside the error of the method. No single individual showed a comparable change in more than one of the tests.

PLASMA PROTEINS. In all three groups a slight but definite fall in plasma proteins was observed which was greater in the men than in the women, and slightly larger in Group III than Group II. This happened in spite of the fact that the protein intake during the working period was estimated to be as high as 85–130 g. daily and the total caloric value of the diet at 2800–3800 calories.

VITAMIN A. Thirty-five subjects examined. Three had initial vitamin A levels below 60 I.U. per 100 ml. plasma. After exposure eight different subjects had similar low values. Six showed an increase of 20 I.U. and twelve a decrease of the same magnitude.

COCARBOXYLASE. Thirty-seven subjects examined. Ten had initial values of 2 mg. per 100 ml. blood. After exposure five, of whom four had initial low values, had similar amounts. Seven subjects showed an increase of 1 mg. per 100 ml. blood and two had a decrease of a similar amount.

VITAMIN C. Where possible, estimation of vitamin C in the white layer of spun blood (mg. vitamin C per 100 g. white layer) and plasma (mg. vitamin C per 100 ml. plasma) were made. Thirty-seven subjects were examined; twenty-six showed no change, five had an increase of more than 5.0 mg. per 100 g. white layer, or more than 0.5 mg. per 100 ml. plasma, and five had a similar decrease. One subject had a critically low value at the initial examination, but it became normal after exposure and no one had a critically low value after the working period.

It was an oversight that the erythrocyte sedimentation reaction was omitted, for there is evidence that

this may be raised after exposure to TNT. (Noro, 1941.)

Discussion

There were significant changes in the blood picture in over 80 per cent. of the students. The haemoglobin was depressed by 10–15 per cent. and there was a mild orthochromic anaemia with an increase in the level of reticulocytes, a tendency to icterus of the haemolytic type, the appearance of urobilin in the urine, and in the more severe examples, hyperplasia of the bone marrow. The anaemia was slight and might well be overlooked in a survey of factory workers, unless careful controls were taken. At the end of the experiment the haemoglobin levels in our students were still higher than those commonly observed in war-time England. The reticulocytosis also was not very great during the period of actual exposure but it became more evident when the worker left contact with TNT. Noro (1941) made an identical observation on the reticulocytes of TNT and tetryl workers in Finland during the Russo-Finnish war. These observations suggest that reticulocytosis is a reparative phenomenon which is to some extent inhibited by the TNT. Thus increase of haemolysis and depression of the haemoglobin values are accompanied by some degree of interference with haemopoiesis. We are therefore justified in concluding that TNT has a haemolytic effect on the majority of those brought in contact with it. The effects are disguised to some extent by the successful response of the marrow, but no haematologist could ignore the changes in the blood and the bone marrow after exposure to TNT.

These results may excite surprise, for opinion in this country has been much influenced by the work of Panton (1917). Panton examined 50 female TNT workers. The average values in 24 women who had been at the work more than three months were: red cells, 5,200,000 per c.mm.; haemoglobin, 88.7 per cent.; in 26 women who had been engaged less than three months: red cells, 5,700,000 per c.mm.; haemoglobin, 90 per cent. There seems to be no doubt that these women were in contact, for their urine gave a positive Webster reaction and some were cyanosed. Moreover, 20 per cent. of another 100 women examined at this factory had "cholaemia," of the serum, which was tinged with bile as judged by naked eye examination. On the other hand, Minot (1919) came to the conclusion that TNT resulted in increased destruction and increased formation of red blood cells. His experiment invited criticism because the tests were performed by medical students, and the results in only 223 of the 600 workers examined were published, no reason being given for the omission of the remainder. The haemoglobin was not estimated and Minot based his opinion on changes in the shape and size of the red cells. Minot's conclusions, therefore, failed to convince other workers in the field.

Nevertheless, subsequent work in man and animals has shown that Minot was right. This work has been ably reviewed by Noro (1941) and we need only repeat the bare facts here. Animal studies have been extended by Himsworth and Glynn (1942) who have demonstrated that chronic TNT poisoning in rats produces a fall in the haemoglobin content of the blood; an erythroblastic reaction of the bone-marrow associated with the appearance of normoblasts, reticulocytes and polychromatic cells, and also of polymorphonuclear cells in the peripheral blood; and an increased deposition of iron in the spleen. The changes in the blood cells, the marrow and the spleen are in general more pronounced in rats on a fat diet, but also occur to a lesser extent in animals on carbohydrate and protein diets. Voegtlin, Hooper and Johnson (1922) found anaemia in 72 per cent. of 237 workers in a TNT factory. It was characterized by a reduction in haemoglobin percentage, the presence of anisocytosis and poikilocytosis, polychromatophilia, fragmentation of red cells, and the appearance of nucleated and reticulated cells in the circulating blood. Noro (1941) found anaemia in 17 out of 38 workers in TNT and a similar incidence in workers in tetryl. He drew particular attention to the high colour index and the frequent occurrence of thrombocytopenia and increased rate of erythrocyte sedimentation. Davie (1942) found that the reticulocyte count in TNT workers was double the normal average.

The unexpected findings that the students working in the melt houses were more severely affected than those in the filling shops, raises the question of the routes of absorption of TNT. Benjamin Moore (1917) in the last war came to the conclusion that the only important route was the skin. In the melt houses the temperature at platform level is high and the TNT is present in the form of fume or vapour. If it is true that exposure under these conditions produces a more acute effect than in the filling shops, all that we are justified in concluding is that a high temperature favours the absorption of TNT. Few chapters of science are more convincing than Moore's demonstration of the preponderant rôle of the skin in the absorption of TNT. Nevertheless opinion is gaining ground that workers absorb TNT by the lungs and the alimentary tract and the changes in the blood and blood-forming organs in the young men in the melt houses were severe enough to suggest the need for further experiments to determine the mode of absorption of TNT there.

Evidence of liver damage in our series was slight and not wholly convincing. This suggests that TNT acts on the liver less rapidly or less constantly than on the blood, or else that our tests of liver function are not sensitive enough to be certain of early changes. Probably each of these factors has influenced the results, but more work is required before the point can be decided. Meanwhile, suggestive changes in the plasma phosphatase and

hippuric acid test were observed in 17 per cent. of cases. It may also be argued that the failure to maintain a normal level of plasma bilirubin and a urine free of urobilin in the face of a relatively mild haemolytic anaemia was evidence of an impairment of hepatic function. (Watson, 1937.) The fall in plasma protein, though statistically significant, was small. In the absence of other causes it too may reflect a change in liver function (Weech, 1938). The absence of an increase in coproporphyrin excretion is rather surprising in view of the blood changes.

To return to the point of departure of this investigation, it would appear that the period of exposure was too short to follow to a conclusion the initial reactions to exposure to TNT. The average stay at the factory was a little less than five weeks, whereas the incidence of TNT jaundice rises to a peak at 15-16 weeks. It is therefore possible that the haemopoietic reaction might have been modified and impairment of liver function and porphyrin metabolism might have been detected if the experiment had been continued longer. The haematological changes are presumably due to increased haemolysis and attempts at compensation by the bone-marrow. It is probable that after a time tolerance is acquired and haemopoiesis returns to a normal level. These are possibilities which we hope to work out by a more continuous and prolonged study of a few individuals. In the early stages, at any rate, TNT exerts a deleterious effect on the blood in man. It inhibits the marrow as well as increasing the destruction of the circulating red cells. Only when the subject is removed from exposure is the marrow able to exert its full power of repair and produce a maximal reticulocyte response. It is therefore not surprising that a refractory or aplastic type of anaemia develops in isolated cases of exposure to TNT.

As regards the value of laboratory tests, the estimation of haemoglobin, red cells, reticulocytes and serum bilirubin gives useful information about the effects from TNT during the early period of exposure. Unfortunately reticulocytosis is not so conspicuous during exposure as it is after 48 hours or more off contact. Noro's work suggests that the sedimentation of the red cells is accelerated when TNT is absorbed. On the other hand, tests of liver function are too time-consuming and the results too uncertain to be of much value as routine procedures in the early weeks. There is no correlation between the Kennedy-Ingham reaction and the excretion of coproporphyrin, and the significance of the former reaction remains to be decided. On the other hand, the direct indican reaction in the urine is so frequently positive in TNT sickness that it might prove valuable as a confirmation of this diagnosis. Anaemia, reticulocytosis, bilirubinaemia and direct indicanuria are pathological phenomena and they provide criteria with more meaning than the Webster reaction and similar urinary tests, which merely signify the excretion of TNT or its derivatives.

Summary

Sixty-two undergraduates were sent to a filling factory for periods varying from four to eleven weeks. During this time they obeyed the same rules and regulations as the regular operatives; received the same rates of pay; and were housed in one of the government hostels for factory workers. Most of the students remained in good health throughout the period of their work.

Immediately before and after exposure a series of tests were carried out in the Biochemical Laboratory of the Radcliffe Infirmary and the Oxford Nutrition Survey. The tests were designed to investigate the effects of TNT on the haemopoietic system, the liver and the general nutrition. These tests were of a detailed type in order to determine any possible early actions of TNT.

Four subjects who experienced abdominal pain were examined with a gastroscope. The gastric mucosa showed mild catarrhal effects.

In 85 per cent. of cases the level of haemoglobin and circulating red blood cells was reduced, indicating that, under the conditions of exposure, TNT acted as a haemolytic agent. The bone-marrow responded to this destruction by a heightened erythropoiesis and an increase in the number of reticulocytes in the circulation; the highest reticulocyte levels were attained after removal from contact.

A significant increase in the plasma bilirubin occurred in 20 per cent. of cases; the greater part was in the direct form.

There was no alteration in the laevulose tolerance test in the series as a whole but in four cases the plasma phosphatase was over fifteen units after exposure and in five the excretion of hippuric acid was reduced by 15 per cent or more.

There was a small but significant fall in the plasma proteins after exposure in over 70 per cent. of the students. A few subjects showed a fall in the vitamin A content of the blood, but there was no appreciable change in the cocarboxylase or vitamin C.

Haemolytic effects were more marked in the men than in the women, and were greatest in the group working in melt houses, where the room temperature and the concentration of TNT fumes was greater than in the filling shops.

The reticulocyte count, the erythrocyte sedimentation rate and the direct indican reaction in the urine are simple tests which merit further evaluation in the control of harmful exposure to TNT. During the first weeks on contact they appear to be of greater clinical signifi-

cance than the Webster reaction, the Kennedy-Ingham reaction or the excretion of coproporphyrin.

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