ON THE METABOLISM OF SOME AROMATIC NITRO COMPOUNDS BY DIFFERENT SPECIES OF ANIMAL

PART III. THE TOXICITY OF THE DINITROPHENOLS, WITH A NOTE ON THE EFFECTS OF HIGH ENVIRONMENTAL TEMPERATURES

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An account is given of an investigation of the toxicity of the dinitrophenols to small laboratory animals. A new pattern of toxicity to chemical structure has been discovered and is discussed in relation to other dinitro-compounds. Also the effects of high temperatures on the toxicity of the dinitrophenols have been studied.

THE basic structure of 2:4-dinitrophenol is present in 2:4-dinitro-*o*-cresol (DNC) and a number of similar compounds widely used as herbicides in agriculture. Because all these substances are poisonous to man and to animals it is desirable to seek alternatives that are less toxic. Considerable information exists on some of the substances derived from 2:4-DNP (e.g., Lawford, King and Harvey[1]), but information on its isomers is limited.

Some years ago Magne, Mayer and Plantefol[2] described the main effects of these substances on dogs, but examination of their results showed that further studies would be necessary to supply information on acute toxicities to small laboratory animals at different temperatures, elimination rates from the blood as indications of their possible accumulation in the body, and stimulation or depression of the oxygen consumption. One result of the present study was to reveal a new pattern of chemical structure to toxicity. This bears some resemblance to that proposed by Magne and colleagues, but it also differs from it in two marked respects. This paper describes this relationship, and the results of the other tests mentioned above.

The Preparation, Relationships and Properties of the Dinitrophenols

All six isomers are readily accessible, and three at least are available commercially (2:4-DNP, 2:5-DNP and 2:6-DNP). 2:4-DNP is prepared by the direct nitration of phenol, and no other isomers appear as by-products[3]. 2:4-DNP and 2:6-DNP are prepared by sulphonation and nitration of *o*-nitrophenol, and 2:3-DNP, 2:5-DNP and 3:4-DNP by the nitration of *m*-nitrophenol[4,5]. 3:5-DNP is prepared by replacement of one nitro group by methoxyl in *sym*-trinitrobenzene[6] and demethylation of the dinitroanisole by anhydrous aluminium chloride[7]. The relationship of the isomers and their parent substances is shown in Figure 1 and the main properties in Table I. All the isomers are insoluble in cold water, and some are volatile in steam[5]. They form bright yellow or orange salts soluble in methyl ethyl ketone. This property has been used by Parker[8] for the analysis of 2:4-DNP and DNC in biological fluids.

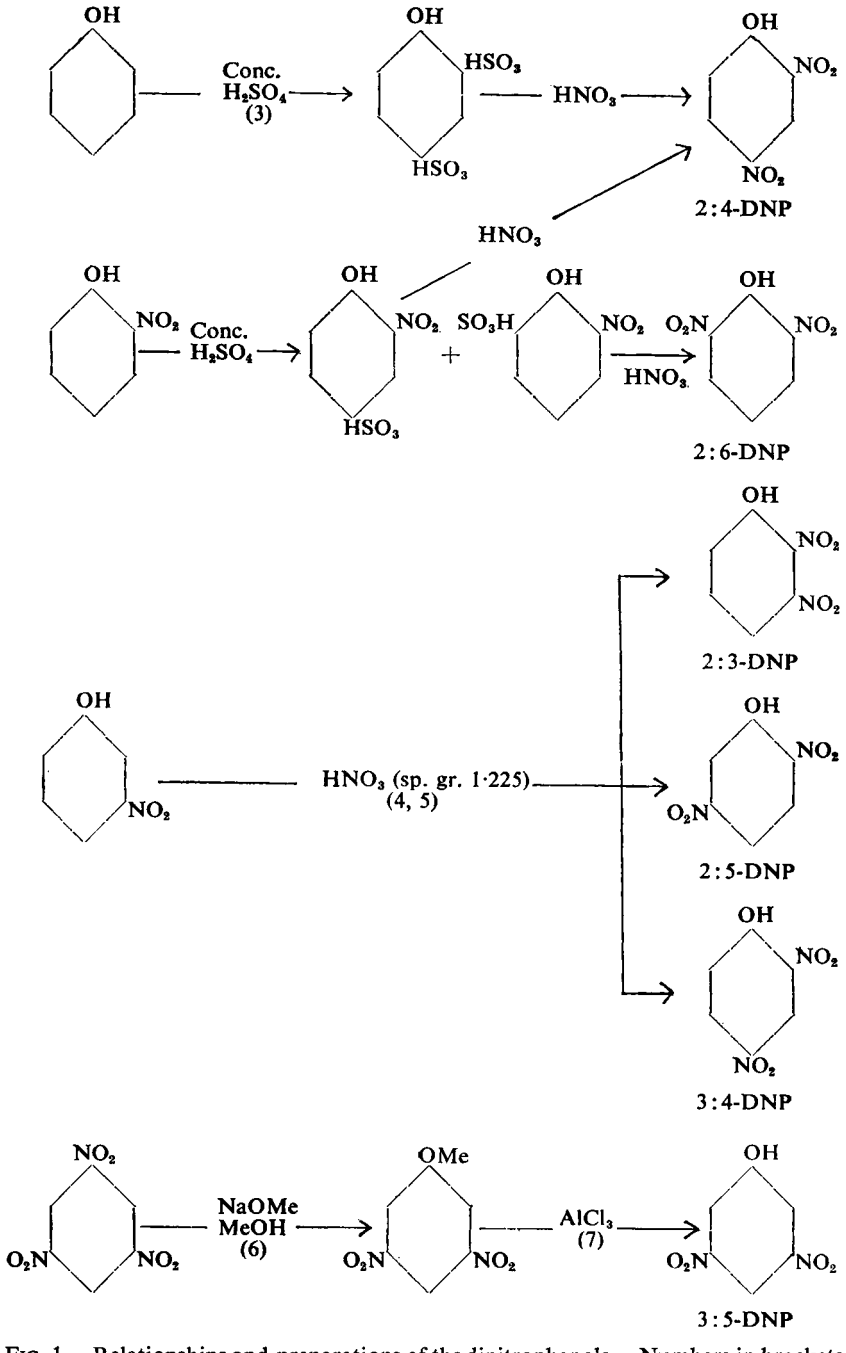

FIG. 1. Relationships and preparations of the dinitrophenols. Numbers in brackets are references.

Materials and Methods

Preparation of the Isomers

2:4-DNP, 2:5-DNP and 2:6-DNP were obtained commercially, and the remainder prepared by the methods outlined above. Methyl derivatives were also prepared and the melting points of these and of the parent dinitrophenols checked with those given in the literature[9].

Nitrogen Determination and Solubilities

The micro-Kjeldahl technique was used as an alternative method for determining solubilities. Since it is well known that sulphuric acid digestion of aromatic polynitro compounds gives low and variable recoveries of nitrogen, experiments on preliminary reduction were carried out to overcome these difficulties. As a result of these the following method was devised.

To a solution of the dinitrophenol in sodium hydroxide (2–3 mg. in sodium hydroxide, 10 per cent w/v, 1·0 ml.) was added sodium dithionate (50–100 mg.). After 1·5 hours the dark coloured fluid was boiled (1·0 min.), cooled and treated with sulphuric acid (50 per cent v/v, containing selenium dioxide, 2·0 per cent w/v, 2·0 ml.) and digested in the usual way (3·0 hours). The digest was distilled with alkali using a Markham apparatus, the ammonia trapped in boric acid (2 per cent w/v, 5·0 ml.) and titrated with sulphuric acid (0·01N) using a screened indicator. Recoveries of nitrogen from standard solutions of the dinitrophenols in alkali were good. The average recovery from eleven determinations on the six isomers was 100·6 per cent, with a range of 98–106 per cent. The solubilities of the isomers determined by this method are given in Table I.

Chromatographic Separation

During the preparation of the isomers preliminary attempts were made to check their homogeneity by one dimensional paper chromatography. These were partially successful and are shown in Table I. No special precautions were taken to control temperature which ranged from 17–20°.

Estimation of Dinitrophenols

Parker's method (*loc. cit.*) was employed using a Unicam Spectrophotometer and a Hilger "Spekker". Ilford Filter 601 proved to be satisfactory for use with the "Spekker" although recoveries differed for the six isomers. The efficiency of the method for the six compounds in decreasing order was found to be 3:4-DNP; 2:4-DNP; 2:6-DNP; 2:3-DNP; 2:5-DNP; 3:5-DNP.

LD50 Determinations

These were made at 18–20°, 35–37°, 39–41°, representing moderate, warm and hot summer day temperatures, by methods already described for other dinitro-aromatics[10].

Solutions of the dinitrophenols in the requisite quantity of alkali were administered by intraperitoneal injection. Before, during, and after

TABLE I

SOME PROPERTIES OF THE DINITROPHENOLS

Dinitrophenol description —OH = 1	General properties: Crystal form	m.p.	k (25°)	Solubility in in water 35–36° g./100 ml. (*a*)	m.p. of dinitroanisole	Chromatography (R_F values): *n*-Butanol, acetic acid, water 4:1:5	*n*-Butanol saturated 5N ammonia	Benzene, acetic acid, water 1:1:2 (*b*)	Colour of alkaline spot (*c*)
						Ascending: 20 hr.	Descending: 5 hr.	Ascending: 3·5 hr.	—
2:3-	Yellow monoclinic prisms	144°	$1{\cdot}3 \times 10^{-5}$	0·22	119°	0·95	0·54	0·83	Red-orange
2:4-	Yellow rhombs or needles	114–5°	$1{\cdot}0 \times 10^{-4}$	0·079	94° (89°)	0·90	0·55	0·95	Yellow
2:5-	Yellow needles	104°	$0{\cdot}7 \times 10^{-5}$	0·068	97°	0·92	0·57	0·95	Red-orange
2:6-	Yellow rhombs	63·5°	$2{\cdot}7 \times 10^{-4}$	0·042	117·5° (118°)	0·86	0·49	0·95	Deep yellow
3:4-	Pale brown or colourless prisms	134°	$4{\cdot}3 \times 10^{-6}$	0·23	70°	0·93	0·59	0·47	Pale yellow
3:5-	Colourless monoclinic prisms	122–3°	$2{\cdot}1 \times 10^{-4}$	0·16	105–6°	0·92	0·60	0·69	Lemon yellow

Notes:

(*a*) The determination was made on a solution of the dinitrophenol prepared by shaking up an excess with water and allowing to stand in warm room for 48 hr.
(*b*) This must be freshly prepared otherwise very streaky runs obtained.
(*c*) Air dried papers held in ammonia vapour.

administration the animals were maintained on normal diets and water *ad lib.*

Elimination rates from the blood. These were determined on mice, rats and rabbits by methods previously described[11].

Oxygen consumption. Following general procedures recommended in a previous paper[12] the effects of sub-acute and low doses were assessed on groups of rats and guinea pigs. Six animals were used for each dose level, and estimations were made 1·0–1·25 hours after administration of the dinitrophenol. Measurements were made on a "group" and not on an individual basis. One four point assay of DNC was made on guinea pigs (Fig. 2).

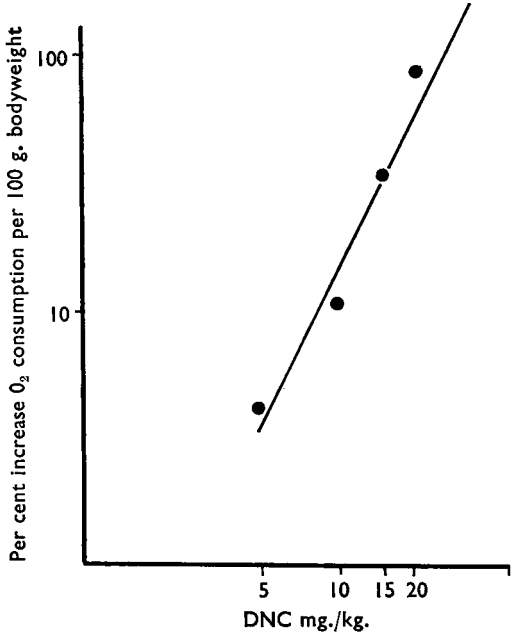

FIG. 2. Oxygen consumption of guinea pigs—average weight of group 1500 g., in response to varied doses of DNC given intraperitoneally. Measurements taken 1·0–1·25 hours after injection. Line by observation.

Body temperatures. These were determined rectally on rats after single sub-lethal doses administered intraperitoneally after three normal readings had been taken at approximately half hour intervals (Fig. 3).

Protection Against the Effects of High Environmental Temperatures

Mice and rats were given a wide range of dose levels of DNPs and DNC and placed in hot room at the highest of the three temperature ranges (37–41°). Varying treatments were then given to animals dosed with dinitrophenols having known stimulant effects (2:4-DNP and DNC) to

investigate the possibility of countering or reducing the exacerbating effect of heat on their toxic activity. The treatments included sponging the animals with water and allowing them continuous contact for 5–6 hours with a little water at the bottom of their cages, immediately, and 1 hour after intraperitoneal injection of the dinitrophenol, parallel and delayed treatment with 4-methyl-2-thiouracil, removal of poisoned animals to a cold room (7°) and treatment with three of the less toxic dinitrophenols (2:3-DNP, 2:5-DNP and 3:4-DNP) with known hypothermic properties. With the exception of those animals removed to the cold room the remainder were returned immediately to the hot room after the other treatments.

TABLE II

LD50 VALUES OF DINITROPHENOLS FOR RATS AND MICE AT THREE TEMPERATURE RANGES, WITH MLD VALUES FOR DOGS (MAGNE AND COLLEAGUES *loc cit.*)

	mg./kg.				
	Rats	Mice	Mice	Mice	Dogs
Dinitrophenol	18–21°	18–21°	35–37°	39–41°	Room temp. (?)
2:3-	190	200	>160<175	>160<175	1000
2:4-	35	36	35	<5 (*a*)	30
2:5-	150	273	∿250	∿200	100
2:6-	38	45	37	<10 (*a*)	50
3:4-	98	112	∿115	>100<110	500
3:5-	45	50	47	50	500

Note.—(*a*) At these dose levels the mortalities were 100 per cent.

TABLE III

COMPARISON OF LD50 VALUES OF DINITROPHENOLS (LD50 FOR 2:4-DNP=1)

Dinitrophenol	Rats	Mice
2:3-	5·4	5·5
2:4-	1·0	1·0
2:5-	4·3	7·6
2:6-	1·1	1·3
3:4-	2·8	3·1
3:5-	1·3	1·4

RESULTS

Acute Toxicity at Three Temperature Ranges

The summarised results showing LD50 values are given in Table II. A comparison is made also with the limited results obtained on dogs by Magne and colleagues. In view of the well-known action of high temperature in increasing the toxicity of DNC[13–15] it was not surprising that 2:4-DNP behaved similarly. Of the remaining isomers only 2:6-DNP showed a definite increase in toxic activity. Even moderately high temperatures (35–37°) have little effect on the toxicity of 2:4-DNP, although a few degrees higher, the response is very much increased. This suggests

TABLE IV

ELIMINATION RATES OF DINITROPHENOLS FROM THE BLOOD OF MICE AND RATS FOLLOWING A SINGLE LARGE DOSE GIVEN INTRAPERITONEALLY

	Mice					Rats				
Dinitrophenol	Dose mg./kg. (*a*)	Sampling times (min.)	μg. Dinitrophenol/g. blood		Half time of elimination (min.) (*b*)	Dose mg./kg. (*a*)	Sampling times (min.)	μg. Dinitrophenol/g. blood		Half time of elimination (min.) (*b*)
			Range	Mean ± SD				Range	Mean ± SD	
2:3-	90	7	33–74	47 ± 16	2·7	90	15	70–136	104 ± 22	12·5
		12	8–16	13 ± 3			30	31–107	68 ± 32	
							60	4–13	7 ± 3	
2:4-	20	30	32–37	34 ± 2	54·0	20	180	29–39	34 ± 3	225·0
		60	21–33	28 ± 4						
		90	9–14	12 ± 2			600	7–11	8 ± 2	
2:5-	180	6	33–60	47 ± 13	3·3	90	7	8–23	14 ± 6	13·0
		12	9–15	13 ± 4			12	8–11	9 ± 2	
							40	2–4	3 ± 1	
2:6-	30	15	49–71	62 ± 8	238·0	25	15	64–76	71 ± 10	210·0
		120	28–51	43 ± 10			150	36–58	50 ± 9	
		240	10–38	21 ± 11			450	10–26	19 ± 9	
3:4-	60	5	38–55	47 ± 9	3·5	90	10	41–64	51 ± 11	11·5
		10	9–23	18 ± 6			40	1–5	4 ± 2	
3:5-	30	4	44–61	56 ± 11	2·7	30	5	22–90	61 ± 30	2·1
		8	5–22	16 ± 8			8	10–19	14 ± 4	

Notes.

(*a*) Dinitrophenol dissolved in the requisite quantity of sodium bicarbonate or sodium hydroxide.
(*b*) Half time of elimination obtained by plotting mean log concentrations against time and reading off time (min.) for the initial (highest) concentration to be halved.

a "critical" temperature point, at least for mice and rats. Temperatures of 37–41° are not unknown in the United Kingdom, and are certainly common in the tropical countries where DNC may be used as an anti-locust measure.

A comparison of the relative toxicities of the dinitrophenols taking 2:4-DNP as unity is given in Table III. This suggests that on an acute toxicity basis the six isomers may be divided into two equal groups: most toxic—2:4-DNP, 2:6-DNP and 3:5-DNP (*meta* relation of both NO_2 groups common to these isomers); least toxic—2:3-DNP, 2:5-DNP and 3:4-DNP (*meta* nitrophenol group common to these isomers).

TABLE V

RATIOS OF LD50 VALUES OF DINITROPHENOLS TO THEIR HALF-TIMES OF ELIMINATION (× 100) FROM THE BLOOD

Dinitrophenol	Rats	Mice
2:3-	6·6	1·5
2:4-	642·8	154·2
2:5-	8·7	1·2
2:6-	552·6	528·8
3:4-	11·7	3·1
3:5-	6·0	4·2

Elimination Rates from the Blood

These are summarised in Table IV and the ratios of LD50 values to the half-times of elimination (× 100) in Table V. The rabbit detoxicates some of the dinitrophenols (2:3-DNP, 2:5-DNP, 3:4-DNP and 3:5-DNP) very rapidly, so much so that none could be detected in the blood 15 minutes after intravenous injection. This great ability to destroy "unnatural" chemicals is not uncommon with this animal which detoxicates and destroys even a proportion of DNC.

TABLE VI

EFFECT OF DINITROPHENOLS ON THE OXYGEN CONSUMPTION OF RATS AND GUINEA PIGS. MEASUREMENTS TAKEN 1·0–1·25 HOURS AFTER INTRAPERITONEAL INJECTION. (GROUP VALUES, SIX ANIMALS PER DOSE)

Dinitrophenols	Per cent increase in oxygen consumption per 100 g. body weight per min.					
	Rats			Guinea pigs		
	10	20 mg./kg.	100	10	20 mg./kg.	100
2:3-	−16	—	+ 3	+ 1	—	+ 4
2:4-	+17	+21	—	+25	+37 (*a*)	—
2:5-	− 7	—	23	− 5	—	+ 4
2:6-	− 2	+ 2	—	0	+13	—
3:4-	− 4	—	−10	< 1	—	+ 8
3:5-	− 5	−20	—	+18	+19	—

Note.—(*a*) Compare with results in a previous paper.[12]

Oxygen Consumption

Summarised results are given in Table VI. From these it is seen that the most active stimulant is 2:4-DNP and that of the others only 2:6-DNP and 3:5-DNP show any positive stimulation. The remaining isomers have the opposite effects, since oxygen consumption appears to

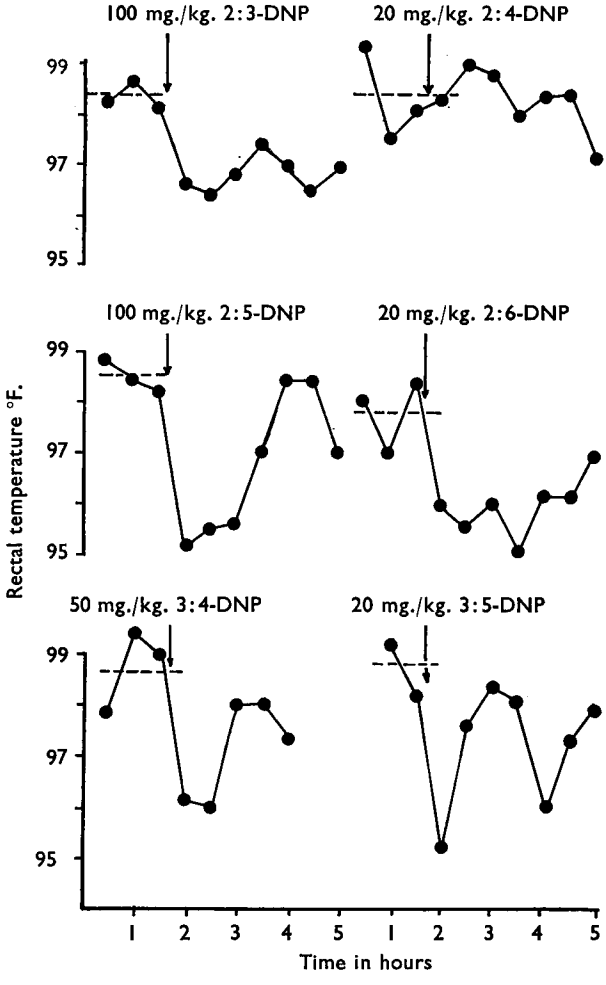

FIG. 3. Rectal temperature of adult rats given single sub-acute doses of the dinitrophenols by intraperitoneal injection after three normal readings.

be retarded, but these differences are insignificant statistically. On the other hand DNC shows a good relation between oxygen consumption and dose, and by simple observation the response appears almost linear. This compared satisfactorily with the results obtained on 2:4-DNP and reported in a previous paper[12]. The statistical analyses of these results will be the subject of a further communication.

Rectal Temperatures

A chronological diagram of the results is given in Figure 3, from which it is seen that only 2:4-DNP is hyperthermic, the remainder all show an initial hypothermia, that diminishes and then increases. This curious double "trough" is a somewhat analogous effect to the reverse and well known phenomenon in which isolated pyrogens (lipopolysaccharides) of bacterial origin cause a double "peak" in an animal's temperature response[16,17].

Protection Against the Effects of High Environmental Temperatures

The protective effect of cooling the animals with water immediately, or 1 hour after administration of 2:4-DNP or DNC is spectacular (Tables VII and VIII). Three of the dinitrophenols and 4-methyl-2-thiouracil were less able to protect, and removal of the animals to a cold room preserved the lives of animals dosed at 10 mg./kg. but not those at 20 mg./kg.

TABLE VII

PROTECTIVE ACTION OF VARIOUS MEASURES AGAINST THE EFFECTS OF HIGH TEMPERATURES ON MICE POISONED WITH DNC AND 2:4-DNP. (39–41° AND 35–37°)

Dose DNC mg./kg.	Mortality per cent over 6 hrs								
	Hot room only 39–41°	Sponging and contact with water		4-Methyl 2-thio-uracil (*a*)	Hypothermic dinitrophenols immediately after DNC			2:4-DNP	
		Immediately after injection of DNC	1·0 hr. after injection of DNC		2:3- at 100 mg./kg.	2:5- at 100 mg./kg.	3:4- at 75 mg./kg.	35–37°	39–41°
2·5	50	0	0	—	—	—	—	0	50
5·0	100	0	0	—	—	—	—	0	100
10·0	100	0	0	60	60	100	50	0	100
20·0	100	10	30	60	100	100	100	0	100
30·0	100	80	70	—	—	—	—	10	100

Note.—(*a*) 10–15 mg./kg. 4-methyl-2-thiouracil intraperitoneally immediately after injection of DNC.

TABLE VIII

PROTECTIVE ACTION OF VARIOUS MEASURES AGAINST THE EFFECTS OF HIGH TEMPERATURES ON RATS POISONED WITH DNC (39–41°)

Dose DNC mg./kg.	Mortality per cent over 6 hr.				
	Hot room only	Sponging and contact with water		4-Methyl 2-thiouracil (*a*)	Removed to cold room (7°) 1·0 hr. after injection of DNC
		Immediately after injection of DNC	1·0 hr. after injection of DNC		
2·5	50	0	0	—	—
5·0	100	0	0	50	—
10·0	100	0	0	100	0
20·0	100	60	100	—	100
30·0	100	100	100	—	—

Note.—(*a*) 10–15 mg./kg. 4-methyl-2-thiouracil intraperitoneally 1·0 hour after injection of DNC.

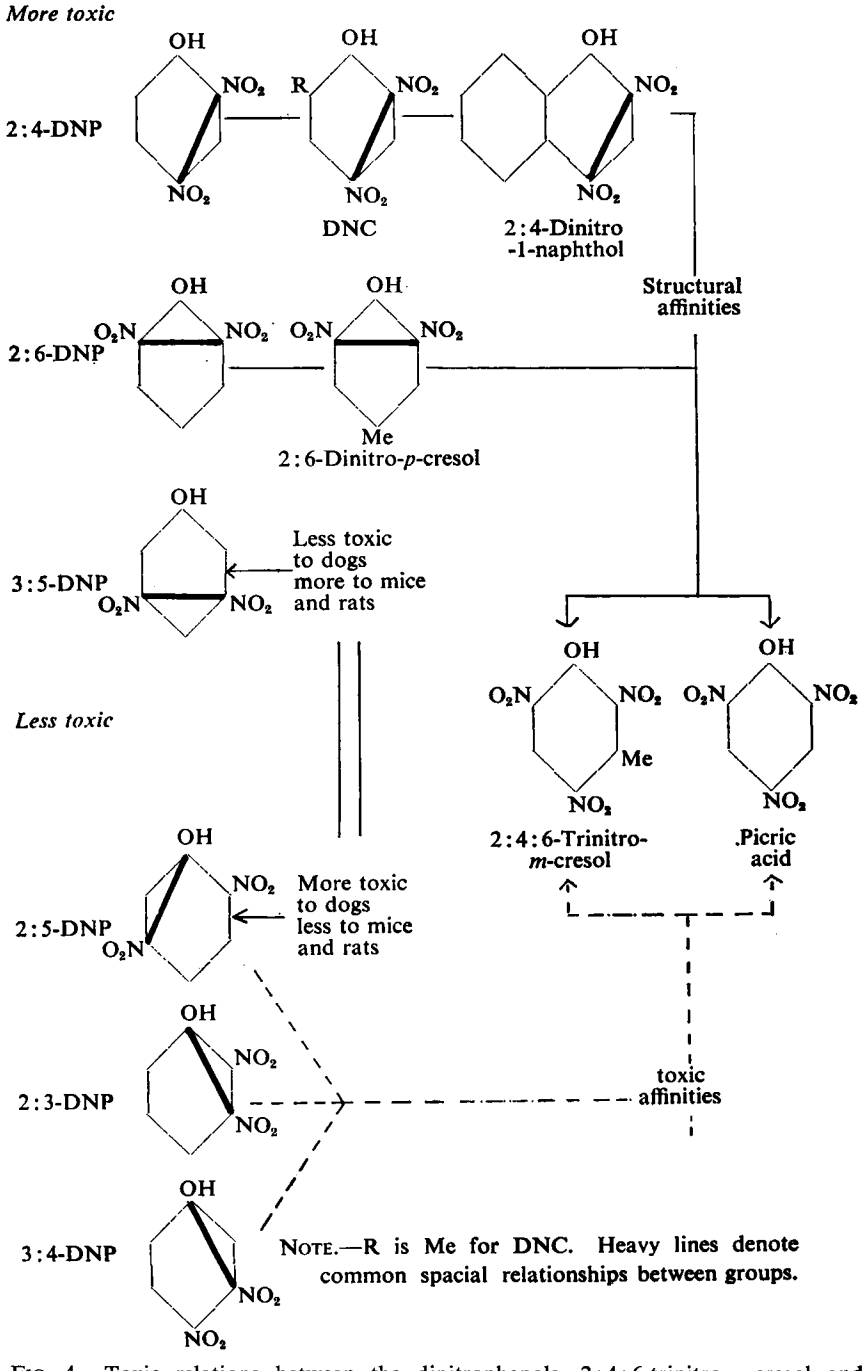

FIG. 4. Toxic relations between the dinitrophenols, 2:4:6-trinitro-*m*-cresol and picric acid.

Discussion

There seem to be alternative patterns of structure to chemical toxicity in the dinitrophenols, and studied in conjunction with the work of Magne and his colleagues there is a hint of an even wider structure-species relation in this interesting group of isomers.

The toxic and structural relationships of the alternatives is shown in Figure 4. The main difference between the two is the reversal in activity between 2:5-DNP and 3:5-DNP. The latter is far more toxic to small laboratory animals than the former, but on the other hand it shares certain common activities with the less toxic groups in both schemes. Thus, it is hypothermic (c.f. Magne and colleagues), it is rapidly eliminated from the blood, and its activity is not increased by high temperatures. The mode of action therefore remains unexplained.

Included in Figure 4 are the cresylic analogues of 2:4-DNP and 2:6-DNP (DNC and 2:6-dinitro-*p*-cresol) and also two closely related trinitro compounds, picric acid and 2:4:6-trinitro-*m*-cresol. From the various experiments reported it can be concluded that addition of an "inactive" group such as methyl, *sec*-butyl or hexyl to the basic 2:4-DNP and 2:6-DNP nucleus will produce relatively minor changes in their quantitative toxicity to man and animals. 2:4-Dinitro-1-naphthol has similar effects to 2:4-DNP. The addition of an "active" nitro-group will alter the degree and nature of the toxicity considerably.

Although the stimulant effects of heat on the action of DNC, 2:4-DNP and 2:6-DNP are severe, the benefits of sponging and contact with water are equally evident. The success of this treatment of severely poisoned rats and mice can not yet readily be explained. A decrease in the environmental temperature alone seems unlikely, because temperatures recorded in the wet cages soon reached the levels of those in the room and in the dry cages. How far skin cooling plays a part remains to be determined, and it is interesting to note that in rats severely poisoned by DNC, the head and shoulders become moist, even though no sweat glands can be identified. It should be remembered that while rats and mice excrete DNC relatively rapidly[15], man excretes it slowly, and therefore sponging and cooling treatment, to be useful, would have to be prolonged and efficient.

The effect of heat in increasing by many times the toxic action of two of the isomers and of DNC, and the very slow excretion by man of the latter, emphasises the need for treating all these substances and related compounds with extreme care, and to apply the proper precautions in handling them.

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