

tuberculosis or cancer, were discharged as a result of an acute respiratory illness contracted during their recruit training.

We are indebted to the P.M.O. Technical Training Command, R.A.F., and other medical officers for granting us facilities to conduct these studies; to many station medical officers and in particular to Wing Commander M. Robertson, Wing Commander M. O. Richardson, and Squadron Leader W. G. Cooke for their co-operation in taking blood samples and completing the special records required; to Wing Commander R. J. A. Morris for help with records of invaliding; to Sergeant A. T. Coukham for valuable technical assistance; and to Dr. P. S. Gardner and Dr. G. B. Bruce White, of the Public Health Laboratory Service, for help with special laboratory tests.

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DINITROPHENOL AND DIABETES MELLITUS

A COMPARISON WITH SALICYLATE

BY

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Salicylate and 2:4-dinitrophenol (D.N.P.) both inhibit oxidative phosphorylation (Loomis and Lipmann, 1948; Brody, 1956). Salicylate also controls the clinical and biochemical abnormalities in moderately severe diabetes mellitus (Reid, Macdougall, and Andrews, 1957). The question that arises is whether D.N.P. has a similar action in this disease.

D.N.P., a powerful metabolic stimulant, was grossly misused in the 1930's as a slimming agent. The principles for prolonged use of a dye substance in clinical medicine put forward by Shannon *et al.* (1944) were not fully appreciated, and the high incidence of serious toxic effects from prolonged administration led to its withdrawal as a therapeutic agent. Since then it has been used only occasionally in clinical practice, although it is still extensively employed in biological investigations.

A short, carefully controlled trial of D.N.P. in diabetes mellitus has been carried out to discover its effect on blood sugar, glycosuria, and glucose tolerance. Six elderly ladies with mild to moderately severe diabetes were investigated along the lines previously described in the aspirin trial. They were given a constant low carbohydrate diet (150 g.) for at least 10 days before starting D.N.P., to establish the effect of diet alone, and the same diet was maintained throughout the whole investigation.

D.N.P. was given for 14 days in doses of 100–300 mg. in capsules daily, actual doses being controlled by

repeated plasma drug levels (Parker, 1949) and by measurement of oxygen consumption of fasting, resting patients with a Benedict-Roth machine to calculate the basal metabolic rate (B.M.R.) (Robertson and Reid, 1952). An attempt was made to induce the same order of metabolic stimulation with D.N.P. as was previously induced in diabetics successfully treated with aspirin. The effects of D.N.P. on fasting blood sugar estimated by Lehmann and Silk's (1952) modification of Folin and Wu's method, on glycosuria determined by Benedict's (1911) method, and on oral glucose tolerance were examined. Since none of the patients had abnormal quantities of ketones in the urine, quantitative measurement was not undertaken.

Results

Dose of D.N.P., Plasma Level, and B.M.R.—Frequent determinations of plasma D.N.P. level and oxygen consumption were necessary to control therapy and to avoid

TABLE I.—Dose and Plasma D.N.P.

Day	Plasma D.N.P. (mg./litre)					
	Case 1 55 yrs. (5)*	Case 2 55 yrs. (52)	Case 3 57 yrs. (2)	Case 4 59 yrs. (96)	Case 5 60 yrs. (12)	Case 6 76 yrs. (12)
1	—	—	21	24	—	15
2	45	40	47	31	40	30
3	45	40	47	31	40	30
4	46	—	54	29	43	44
5	45	—	54	38	41	43
6	49	33	47	—	—	—
7	—	—	—	32	36	32
8	57	36	38	38	45	15
9	—	—	47	43	—	—
10	49	33	42	34	39	48
11	—	48	—	38	—	—
12	45	—	52	40	37	46
13	—	43	—	—	—	—
14	40	50	48	35	41	39
Mean (2–14 days)	47	40	48	35	40	34
Total dose (g.)	2.8	3.1	2.25	3.5	2.9	3.05
Maintenance dose (mg. daily)	150–200	200–300	100–200	200–300	200–300	150–300

* Duration of diabetes in months.

undesirable reactions. Table I gives relevant clinical particulars as well as doses and plasma levels of D.N.P. A fairly steady plasma concentration of D.N.P. was reached in each patient after the first day's dosage; the mean values of the six patients between the second and fourteenth days

ranged from 34 to 48 mg./litre. The relation between plasma D.N.P. and the B.M.R. is shown in Fig. 1: an obvious and expected rise in B.M.R. accompanied an increase in plasma D.N.P. concentration. The regression line and its 95% confidence limits are shown. No toxic effects from D.N.P. were encountered, and the results, in addition to defining a significant relation between plasma D.N.P. and B.M.R., indicate that the same order of metabolic

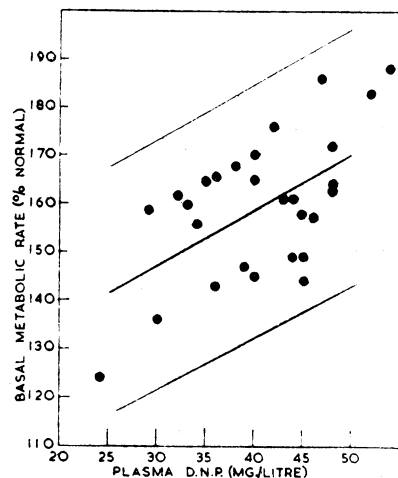


FIG. 1.—Relation between the rise in B.M.R. from D.N.P. and the plasma concentration of the drug in six female diabetics. The regression line and its 95% confidence limits are shown. The coefficient of correlation, r , was 0.54 with $p > 0.01$, but < 0.001 ($t_{0.05} \times \sigma r = 26.5$).

stimulation has been obtained with D.N.P. as in an earlier investigation with aspirin in diabetes mellitus.

Fasting Blood Sugars.—Fasting blood sugars of the six patients at intervals throughout the investigation are shown in Table II. The values of each patient towards the end of the control dietetic period were close enough to one another

TABLE II.—D.N.P. and Fasting Blood Sugar

Case No.	Fasting Blood Sugar (mg./100 ml.)						
	Before D.N.P.		During D.N.P.			After D.N.P.	
	Days 4-5	Days 0-1	Days 3-4	Days 7-8	Days 13-14	Days 3-4	Day 6
1	146	136	158	152	152	—	133
2	157	228	212	202	196	173	208
3	224	250	200	133	124	132	129
4	307	304	214	222	204	185	—
5	325	293	293	289	252	278	—
6	279	262	241	211	190	193	189
Mean ..	240	246	220	202	186	192	—

to indicate that a comparatively stable state had been reached from which the effect of D.N.P. could be judged. In the two weeks that D.N.P. was given the fasting blood sugar of the patient with the mildest diabetes increased a little. The values of the other five patients fell slightly, but in no patient did they reach normal. The group mean concentrations were: 246 mg. per 100 ml. on the day before or on the actual day D.N.P. was started and 186 mg. per 100 ml. after 14 days of D.N.P. administration. The hypothesis that the difference between the mean blood sugars immediately before and at the end of the D.N.P. course was zero was rejected because the t-test gave a value of p between 0.05 and 0.02. The fall in mean fasting blood sugar was therefore just significant.

Glycosuria.—The amount of sugar in the urine was estimated before, during, and after administration of D.N.P., and the results, shown in Table III, agree with the fasting

TABLE III.—D.N.P. and Glycosuria

Case No.	Urinary Sugar (g./24 hours)						
	Before D.N.P.		During D.N.P.			After D.N.P.	
	Days 4-5	Days 0-1	Days 3-4	Days 7-8	Days 13-14	Days 3-4	Day 6
2	12	8	6	3	4	8	9
3	10	10	3	3	0	0	0
4	23	14	12	16	4	0	—
5	39	34	63	36	29	20	—
6	44	32	39	16	17	18	6
Mean ..	26	20	25	15	11	9	—

blood sugars just described. The mildest diabetic had no glycosuria before, during, or after D.N.P. Glycosurias of the other five patients progressively fell during the period D.N.P. was given, but only one patient was sugar-free at the end of the course. The mean fall was from 20 g. of glucose in the 24 hours immediately before D.N.P. to 11 g. at the end of the D.N.P. course. Again the hypothesis that the difference between the mean urinary sugars before and after D.N.P. was zero had to be rejected since p was between 0.02 and 0.01 by t-test. The reduction of glycosuria from D.N.P. was significant.

TABLE IV.—Oral Glucose Tolerance Test

	Blood Sugar (mg./100 ml.)														
	Case 1		Case 2		Case 3		Case 4*			Case 5*			Case 6*		
	Before D.N.P.	After D.N.P.	Before D.N.P.	After D.N.P.	Before D.N.P.	After D.N.P.	Before D.N.P.	After D.N.P.	After Aspirin	Before D.N.P.	After D.N.P.	After Aspirin	Before D.N.P.	After D.N.P.	After Aspirin
Fasting ..	136	152	228	196	224	138	304	204	101	293	289	109	214	190	95
½ hour ..	244	206	288	276	288	185	442	304	193	350	338	138	342	296	164
1 ..	304	248	344	304	324	219	428	363	221	408	408	200	342	304	222
1½ hours ..	292	259	400	328	332	193	372	356	171	428	423	200	384	296	178
2 ..	240	208	364	384	296	159	338	282	143	428	446	240	328	296	143

* Consecutive course of D.N.P. and aspirin given.

Oral Glucose Tolerance.—The blood-sugar values immediately before and at half-hour intervals for two hours after an oral dose of 50 g. of glucose are presented in Table IV. The only difference before and after the course of D.N.P. was that glucose tolerance improved a little in two of the six patients: improvement was slight in one and moderate in the other. In the other four patients there was no noteworthy change.

Body Weight.—The weights of the patients before, at the end of the D.N.P. course, and five days after it had been discontinued are shown in Table V. The weights before D.N.P. were steady. Three patients lost 1.3 to 2.1 kg. in

TABLE V.—D.N.P. and Body Weight

Case No.	Body Weight (kg.)		
	Before D.N.P.	End of D.N.P. Course	Five Days After D.N.P.
1	56.9	55.6	56.5
2	78.0	75.9	75.5
3	79.4	77.5	77.4
4	67.4	67.8	67.6
5	55.1	55.0	54.8
6	42.9	42.2	42.0
Mean ..	63.3	62.3	62.3

the 14 days D.N.P. was given. The weights of the other three were not materially altered. In the five days immediately after D.N.P. there was no significant change, so that, unlike salicylate, discontinuation of D.N.P. is not followed by diuresis and slight weight loss (Reid *et al.*, 1957). No serious weight changes resulted or followed the 14-days course of D.N.P.: the mean weight loss was 1 kg.

The results so far presented suggest that D.N.P. lowers fasting blood sugar, diminishes glycosuria, and may or may not improve oral glucose tolerance without materially altering body weight. In all these respects it resembles salicylate except that salicylate is much more potent and effective. Nevertheless the qualitative similarity has made it necessary to compare D.N.P. and aspirin. Firstly, the effects on fasting blood sugar and oral glucose tolerance of both substances, given consecutively to four patients in the present study, was examined, and, secondly, the changes in fasting blood sugar in the present group of six patients receiving D.N.P. has been compared with those of a previous group treated with aspirin.

Comparison between Aspirin and D.N.P.

Four of the patients given a 14-days course of D.N.P. were treated with aspirin for a similar period. A short interval of a few days elapsed between stopping D.N.P. and starting aspirin, to allow the D.N.P. to be cleared from the body. This was judged by its disappearance from the plasma or by a fall in plasma concentration to a low value. Comparison between D.N.P. and aspirin on fasting blood sugar and on oral glucose tolerance was undertaken. Measurement of urinary sugar was not considered necessary because it followed the blood-sugar changes induced by D.N.P. and aspirin and also because of the difficulty of making proper allowance for the non-glucose reducing substance which appears in the urine during intensive salicylate therapy.

Table VI compares the effect of both drugs on fasting blood sugar; it will be seen that the mean value, 272 mg. per 100 ml. just before D.N.P. fell to 211 mg. per 100 ml.

TABLE VI.—Comparison of Consecutive Courses of D.N.P. and Aspirin

Case No.	Fasting Blood Sugar (mg./100 ml.)								
	Before D.N.P.	During D.N.P.				Before Aspirin	During Aspirin		
	Days 0-1	Days 3-4	Days 7-8	Days 13-14	Days 0-1	Days 3-4	Days 7-8	Days 13-14	
2	228	212	202	196	185	104	104	90	
4	304	214	222	204	185	115	143	101	
5	293	293	289	252	278	268	138	109	
6	262	241	211	190	189	127	118	95	
Mean..	272	240	231	211	209	154	126	99	

after the 14 days' course. The mean fasting blood sugar just before aspirin was 209 mg. per 100 ml. and this fell to 99 mg. per 100 ml. after 14 days' therapy. The fall with aspirin was greater and more rapid than with D.N.P., though the pre-treatment level was lower. When the t-test was applied to the fasting blood sugar before and after D.N.P. the hypothesis that the difference between the means was zero had to be rejected because p was between 0.05 and 0.02. The same hypothesis had to be rejected before and after aspirin, since p was between 0.02 and 0.01.

Oral glucose tolerances with a test dose of 50 g. of glucose were carried out in three patients before and after D.N.P. and then after a consecutive course of aspirin (Table IV). Slight improvement in glucose tolerance was noted in one patient after D.N.P. All three showed pronounced improvement from aspirin, which just fell short of a normal response in two.

These findings indicate that, while consecutive courses of D.N.P. and aspirin both reduce fasting blood sugar, the main difference between them is that the reduction from aspirin is more rapid and complete in that fasting blood sugar returned to normal in all four patients. It did not even approach the normal value in any of the four patients treated with D.N.P. This qualitative similarity but quantitative difference in favour of aspirin was emphasized by the results of the oral glucose-tolerance tests.

The increases in oxygen consumption expressed as basal metabolic rates from the third to fourteenth days during consecutive courses of aspirin and D.N.P. to the same patients have been examined in relation to changes in fasting blood sugar. The results presented in the scatter diagram (Fig. 2) emphasize the difference in potency

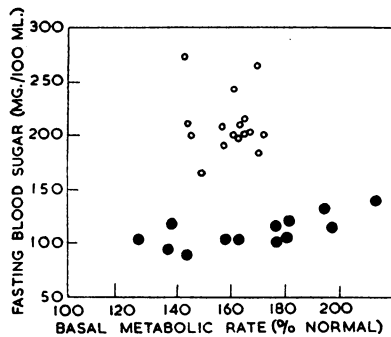


FIG. 2.—Relation between oxygen consumption expressed as B.M.R. and fasting blood sugar during consecutive courses of D.N.P. and aspirin to four diabetic patients is shown. D.N.P. (open circles ○) is associated with abnormally high fasting blood sugars; aspirin (solid dots ●) with normal values for the same increase in oxygen consumption.

increased oxygen consumption in diabetes mellitus from D.N.P. does not reduce the high fasting blood sugar of the diabetic below a certain level, while a similar increase in oxygen consumption from aspirin brings it right down to normal. In both cases the increase in oxygen consumption was as great as would be tolerated in practical therapy.

The superiority of aspirin over D.N.P. in lowering fasting blood sugar may in part be due to the advantage of lower initial fasting blood sugars, since aspirin therapy always followed D.N.P. The importance of this factor has been examined by comparing fasting blood sugars of two groups of six patients treated with D.N.P. or aspirin. One group comprised the six patients in the present investigation who received D.N.P.; the other group was treated with aspirin, and their particulars have already been published (Reid *et al.*, 1957). The patients were all mild to moderately severe diabetics, and on clinical assessment they were comparable. The mean fasting blood sugar just before D.N.P. was 245 mg. per 100 ml.; that before aspirin was 190 mg. per 100 ml. The difference between these means is not significant, since the t-test gave a value of p greater than 0.05 and a preliminary F test indicated that p was more than 0.05. This is consistent with the suggestion that the two groups were clinically comparable before the treatments. The higher value of the mean fasting blood sugar in the D.N.P. group is at least partly accounted for by the greater mean carbohydrate intake of this group over the aspirin group. The actual figures were 150 g. and 130 g. per day respectively.

The mean fasting blood sugars fell during treatment with D.N.P. and aspirin. The fall in the aspirin group, from 190 to 92 mg. per 100 ml. in 14 days, was greater than that in the D.N.P. group, which was from 245 to 186 mg. per 100 ml. in the same period. Aspirin's obvious superiority was borne out by the values of p when t-tests were applied to fasting blood sugar before and after D.N.P. and aspirin. The hypothesis that the difference between the means before and after the two treatments was zero had to be rejected. The value of p in the D.N.P. group was between 0.05 and 0.02; in the aspirin group it was less than 0.001.

Discussion

The fall in fasting blood sugar and the reduction in glycosuria from D.N.P. were not expected. MacBryde and Taussig (1935) reported a slight rise in fasting blood sugar and lowered glucose tolerance in two pre-diabetics and one frank diabetic who were given similar doses of D.N.P. The discrepancy between their findings and ours may be more apparent than real, since one patient in our group, comparable to their pre-diabetics, also showed a slight increase in fasting blood sugar after D.N.P. (Table II). It may be that the milder the diabetes the less likely is the blood sugar to fall, for we have observed that rheumatic patients while receiving full doses of salicylate may show a rise in fasting blood sugar associated with diminished glucose tolerance (Cochran, Watson, and Reid, 1950). Indeed, the action of salicylate in diabetes could not have been predicted from its effect in the non-diabetic, emphasizing once again the danger of premature generalization from incomplete data. No subject is richer in examples of this than the literature on salicylate, where it is almost impossible to make a new discovery, but where so much has still to be correctly interpreted.

The similarity in biological effects between salicylate and D.N.P. is most remarkable. Both increase oxygen consumption of cells and mitochondria, and at the same time reduce uptake of inorganic phosphate (Loomis and Lipmann, 1948; Brody, 1956). This inhibition of oxidative phosphorylation is, in biochemical terms, the basic property shared by them. Sodium salicylate and D.N.P. have the same effect on oxygen uptake by certain tissues with a variety of substrates (Sproull, 1957). Both substances stimulate oxygen consumption in animals to the extent of causing death in hyperpyrexia with high doses (Reid, 1957a). They also inhibit plant growth (Reid, 1957b). In all their similarities over a wide biological field, ranging from wheat coleoptile sections, tissue slices, to whole animals, including man, the effective concentrations remain remarkably constant. The current investigation with diabetes brings out yet another similarity in that both lower fasting blood

sugar and glycosuria, though for the same order of increase in oxygen consumption the effect of D.N.P. is slight compared with that of aspirin, and would in itself have no practical value in the treatment of the disease. This raises the question, Is it enough to say that the fundamental action of both D.N.P. and salicylate is inhibition of oxidative phosphorylation without further specification to account for quantitative differences?

Summary

Salicylate and 2:4-dinitrophenol (D.N.P.) inhibit oxidative phosphorylation. Salicylate also controls moderately severe diabetes mellitus. The effect of D.N.P. in the disease has been investigated.

Increased oxygen consumption from D.N.P. of the same order as is required with aspirin to control moderately severe diabetes lowered fasting blood sugar and glycosuria of six elderly diabetics. The reduction was, however, less than that achieved with aspirin, and would have no practical value in treatment.

This difference between D.N.P. and salicylate in diabetes makes it difficult to account for the action of both substances on the basis of inhibition of oxidative phosphorylation, at least, without further specifications.

My thanks are due to Miss M. McCombie, of the dietetic department of the Western Infirmary, for supervision of the diets, and to the staff of the Unit for invaluable technical assistance.

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A CASE OF CONJOINED TWINS

BY

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Aird's (1954) remark, that owing to the greater accessibility of large groups of populations in Africa and Asia conjoined twins are seen with increasing frequency, is supported by the birth of the Kano and Port Harcourt twins in Nigeria, the case of the twins in Singapore (Roddie, 1957), and our own case as described below.

Case Report

An African woman, gravida-3, about 25 years of age, was seen in the antenatal clinic of our hospital on May 22. She gave a history of 32 weeks' amenorrhoea. On examination the fundus was felt three fingerbreadths under the ensiform cartilage. There was hydramnios, and two hard parts were felt in the fundus. No foetal heart was heard. Twin pregnancy was suspected, and an x-ray film, taken to confirm the diagnosis, showed two foetuses with the heads *in fundo* and the legs well down in the pelvis. The twins were in perfect apposition. Apart from this abnormal position, the vertebral columns showed a peculiar curve, suggesting that the twins were joined at the level of the sternum, thus not allowing the vertebral columns to assume their normal curvature. A presumptive diagnosis of con-

joined twins, with fairly firm union in the region of the sternum, was made, and the woman was admitted.

The next morning an oblique x-ray film was taken. This view showed the details even better, but no definite union could be diagnosed. Several student-midwives were allowed to examine the patient, and a few hours later she com-



Photograph of the conjoined twins.

plained of lower abdominal pains. Very soon the membranes ruptured and a large quantity of blood-stained liquor amni drained away. One foot presented at the vulva. Examination *per vaginam* showed that there was full dilatation.

Having read Wilson Roddie's report on a similar case, I decided to follow the same method, and the patient was taken to the theatre, where she was anaesthetized. The remaining three feet were brought down, one of them causing considerable difficulty. Then the four legs were delivered simultaneously. Hardly any traction was used, the only force being firm expression. The bodies now descended parallel to each other without any difficulty. It became obvious that the twins were joined together, for they had one abdominal cavity, with imperfect closure in the lower part. When the scapulae were well visible, the legs and trunks were lifted right forward over the symphysis pubis. The posterior head engaged and came out without difficulty, immediately followed by the anterior head. Both foetuses were dead and showed signs of maceration.

A bilateral episiotomy was repaired with catgut. The puerperium was uneventful.

The twins, both girls, had a total weight of 2,350 g. (5 lb. 4 oz.). There was a single placenta with velamentous insertion of two cords. The distance between the insertion of the two cords was 2 cm. The placenta showed small infarcts.

Anatomy

The two female foetuses were preserved in formol-saline and examined after a few weeks. They were joined from the manubrium sterni to just under the umbilicus. The union at the level of the sternum was cartilaginous.

Dissection showed that each twin had its own lungs. The right lungs showed only two lobes, although there was an indication of a third lobe (partial fissure). It seemed that they had one heart in common, but after opening of the