Inhibiting Effect of 2 : 4-Dinitrophenol on Calciferol-induced Metastatic Calcification in the Rat

PREVIOUS investigations by Trout[1] and by us[2] indicate clearly that the deposition of mineral in the aortæ of calciferol-intoxicated rats continued long after cessation of administration of this compound and after the blood calcium-level had returned to normal. It appears that, once initiated, this process follows an irreversible course, at least in the aorta, since even 160 days after the initial insult there was no evidence of a reduction in the mineral content of the aorta. Attempts to inhibit calciferol-induced calcification of the aorta by the simultaneous administration of papain (which reduces the mucopolysaccharide content of the aorta) were unsuccessful[3].

Since the calcification is accompanied by a large increase in aortic phosphorus content, it was decided to investigate the effect of 2 : 4-dinitrophenol (DNP) on the action of calciferol administered simultaneously; for it has been shown by Whitehead and Weidman[4] that DNP *in vivo* inhibits the synthesis of adenosine triphosphate, as measured by the incorporation of phosphorus-32, and also the incorporation of the isotope into the bone salt of kitten cartilage. Gutman and Yii[5] found that calcification *in vitro* was markedly inhibited by 2 : 4-dinitrophenol at 10^{-4} *M* concentration. Whitehead and Weidman suggested that the relationship between phosphorus-32 incorporated into bone salt and adenosine triphosphate is that the latter is required to initiate the formation of a primary seed which acts as a nucleus for the subsequent deposition of bone salt.

Accordingly, 20 male Wistar strain rats, initially weighing approximately 250 gm., were divided randomly into four groups of five. Group I was given, twice daily, intraperitoneal injections of 3 ml. of 0·9 per cent sodium chloride solution for twenty days. Group II received identical saline injections, together with calciferol (a total of 100,000 units/kgm. body-weight) given orally, in daily doses, as a solution in arachis oil from day 6 to 10 inclusive. Group III were given, twice daily, injections of 3 ml. of a saturated solution of DNP in 0·9 per cent sodium chloride, while Group IV received the same injections together with calciferol orally as in Group II. On day 20 the animals were killed and the aorta, lungs and stomach dissected out. The tissues were dehydrated with acetone, defatted with ether–alcohol (1 : 1 v/v) and dried to constant weight at 100° C. A second series of animals received the same treatment, but were killed on day 9 of the experiment, that is, 4 days after starting calciferol treatment, and bled from the heart; the serum was analysed for calcium and inorganic phosphorus. Differences between means were assessed by the Mann–Whitney *U* test, using the 5 per cent level of significance.

Table 1 shows the mean aortic dry weight and the calcium and phosphorus contents of the aortæ. Calciferol treatment resulted in an approximately two-and-a-half-fold increase in mean aortic dry weight, similar to that previously found by us[2]. We have found this increase in weight to be due largely to the increased mineral content of the aortæ (see below). For this reason, the results for calcium and phosphorus are expressed in mgm./aorta. It will also be seen from Table 1 that DNP, administered simultaneously with calciferol, significantly reduced the effect of calciferol on total aortic weight, although

Table 1. DRY WEIGHT AND MINERAL CONTENT OF RAT AORTÆ

Group	Treatment	Dry fat-free weight (mgm.)	Calcium (mgm./aorta)	Phosphorus (mgm./aorta)	Calcium/phosphorus ratio
I	Saline only	16·7 ± 1·2*	0·21 ± 0·06	0·07 ± 0·01	3·0: 1
II	Saline + calciferol	43·8 ± 5·5	6·23 ± 0·91	2·30 ± 0·33	2·7 : 1
III	DNP only	16·7 ± 1·0	0·11 ± 0·01	0·08 ± 0·01	1·4 : 1
IV	DNP + calciferol	28·1 ± 4·0	2·88 ± 1·04	1·15 ± 0·31	2·5 : 1

* Mean ± *S.E.*

DNP alone had no effect at all on the aortic dry weight.

Calciferol treatment also resulted in significant increases in both calcium and phosphorus contents of the aorta; DNP alone had no effect on these constituents, but when given simultaneously with calciferol, significantly reduced the degree of mineralization induced by calciferol, by approximately one-half. The calcium–phosphorus ratio of the aortæ tended to be reduced in the two groups receiving DNP injections, but, due to wide variations in individual values, the differences were not significant.

Results similar to those from aortæ were obtained for lung and stomach; for calciferol produced significant increases in the calcium and phosphorus contents of these organs as well[2] and DNP, given simultaneously, reduced this effect.

The results of the serum calcium and phosphate determinations showed that calciferol markedly increased both these serum constituents (calcium–phosphorus product raised to 125 compared with control value of 77). However, despite its marked effects on tissue mineralization, DNP given simultaneously with calciferol did not affect the increases in serum calcium and phosphorus induced by calciferol.

Hence it would appear that the inhibiting effect of DNP on the calcification induced by calciferol is not mediated via any overt action on blood calcium or phosphate-levels. It would thus seem to exert some direct effect on the tissues, although an indirect action, via the endocrine system, for example, parathyroids, cannot be ruled out at present.

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