diac glycosides may be different. Gold and associates have described large differences in convulsant activities of a variety of cardiac glycosides in the rat. They suggested that the variations were due to differences in metabolism or fixation of the glycosides. Ouabain had relatively little convulsant action (10).

The volume of injectate used in each animal for each thermodilution curve was 0.1 ml and total volume for each animal did not exceed 0.8 ml physiological saline. This volume, even if given in one injection, could not explain the responses observed. The vehicle for the ouabain was similarly excluded as the explanation of the cardiac responses.

The thermodilution method in our hands is reproducible within ± 6% (2). The significant changes in cardiac output after administration of ouabain (0.05 to 0.09 mg/kg) were far in excess of this variability. This technic offers the possibility of further study of the mechanism of action of cardiotonic drugs in small animals. It is simple, inexpensive and provides a reliable, experimentally repeatable measurement of cardiac output in the same small animal.

Summary. The cardiac output of anesthetized albino rats increased following administration of ouabain in doses of from 0.05 to 0.09 mg/kg i.v. Mean arterial blood pressure and heart rate were unchanged; calculated total peripheral resistance decreased. Signs of toxicity were not observed over a dose range of from 0.01 to 0.05 mg/kg despite the appearance of the positive inotropic effects.

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Action of Parathyroidectomy and Dihydrotachysterol on Myocardial Calcification Due to Coronary Vein Obstruction.* (28160)

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Extensive investigations during the past few years on various experimental cardiopathies(1) have not provided a satisfactory answer as to why some structural alterations in the myocardium and other tissues constitute a prerequisite for calcification. It is nevertheless true that among the various forms of necrotizing cardiopathies induced in animals, some (*e.g.,* those produced by proteolytic enzymes, neomycin, polymyxin, sulfonamides, nephrectomy) calcify more consistently than others(2). As yet, we are not justified in classifying cardiopathies as calcifying and non-calcifying since the same agent (Plasmocid or paraphenylenediamine), according to the mode of administration and other undetermined factors, elicits degenerative changes within the cardiac or skeletal musculature that may or may not be accompanied by calcific deposits(3,4). A new approach to the problem was provided by the observation that while the massive cardiac infarct that follows coronary artery ligature never calcifies, the less extensive but still

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TABLE I. Prevention by Dihydrotachysterol of Myocardial Calcification Normally Due to Coronary Vein Obstruction.

Treatment*	Final body wt (g)	Cardiac lesions: Necrosis Grade (0-3)	Cardiac lesions: Necrosis Incidence (%)	Cardiac lesions: Calcification Grade (0-3)	Cardiac lesions: Calcification Incidence (%)	Serum electrolytes (mg%): Calcium	Serum electrolytes (mg%): Inorganic phosphorus
None	122 ± 3.6	1.3 ± .19	100	1.2 ± .24	60	10.6 ± .12	11.2 ± .29
DHT	114 ± 2.8	1.2 ± .25	100	.1 ± .1	10	10.8 ± .16	11.6 ± .25
Parathyroidectomy	110 ± 4.2	1.6 ± .27	100	2.1 ± .21	100	7.1 ± .15	15.9 ± .34
" + DHT	104 ± 2.1	1.5 ± .21	100	.4 ± .17	20	9.8 ± .18	10.8 ± .18
Thyroparathyroidectomy	106 ± 1.8	1.2 ± .23	100	1.1 ± .21	80	6.9 ± .17	15.1 ± .29
" + DHT	102 ± 2.6	1.4 ± .25	100	.2 ± .15	10	10.3 ± .23	11.4 ± .23

* In addition to the treatments listed above, in all rats of Groups 1 to 6 the 2 main coronary veins were ligated, as described in text.

marked necrosis resulting from coronary vein obstruction is always heavily filled with calcium deposits(5).

The following is a report of experiments indicating that myocardial calcification that normally follows coronary vein ligature is: 1) greatly enhanced by parathyroidectomy, but not by thyroparathyroidectomy and 2) prevented by administration of dihydrotachysterol (DHT) in intact as well as in parathyroidectomized or thyroparathyroidectomized rats.

Materials and methods. Sixty female rats of the Sprague Dawley Farm, with a mean initial body weight of 105 g (range: 98-112 g), were subdivided into 6 equal groups and treated as indicated in Table I. Dihydrotachysterol, or DHT ("Calcamin," Wander) was administered from the first day throughout the period of observation. Daily dosage by stomach tube was 50 μg per rat in 0.5 ml of sesame oil. Thyroparathyroidectomy and parathyroidectomy were both performed under ether anesthesia on the first day of the experiment, the former by blunt dissection of the thyroid (including the parathyroids), the latter by selectively destroying the parathyroids with the aid of a thermocautery. Ligature of the coronary veins was performed under light ether anesthesia on the 5th day, by using the surgical procedure described elsewhere(5). Here, we simply mention that the 2 main coronary veins were ligated at the point where they enter (separately or after uniting into a single sinus) the right auricle. The experiment was terminated on the 10th day, *i.e.*, 5 days following coronary vein obstruction, and blood was taken for electrolyte determinations. Sera were analyzed individually for inorganic phosphorus, according to the method of Fiske and Subbarow(6), and for calcium by the Coleman flame-photometer technic.

At autopsy, the hearts were first inspected with the aid of a dissecting loupe, then fixed in alcohol-formol for subsequent embedding in paraffin. Three sections prepared from each heart at different levels were stained with the von Kossa technic for histochemical demonstration of calcium, using hematoxylin-phloxine-saffron counterstain. The severity of the myocardial lesions (necrosis, calcification) was assessed on an arbitrary scale of 0 to 3: 0 designating no lesion; 1 just detectable lesions; 2 moderate lesions; 3 severe lesions. In Table I, we list the means of these readings (with corresponding standard errors) as well as the percentual occurrence of the lesions.

Results. Following occlusion of the 2 major cardiac veins (or, in the case of confluence between them, of the resulting terminal sinus itself), a pronounced pericarditis develops over the region drained by the ligated trunks, necrotic foci occur in the subepicardial muscle layers, and massive calcification begins in these affected areas. Macroscopically, this manifests itself in the development of white, isolated or confluent subepicardial spots that form a corona at some distance from and surrounding the occluded veins. Histologically, the affected areas usually

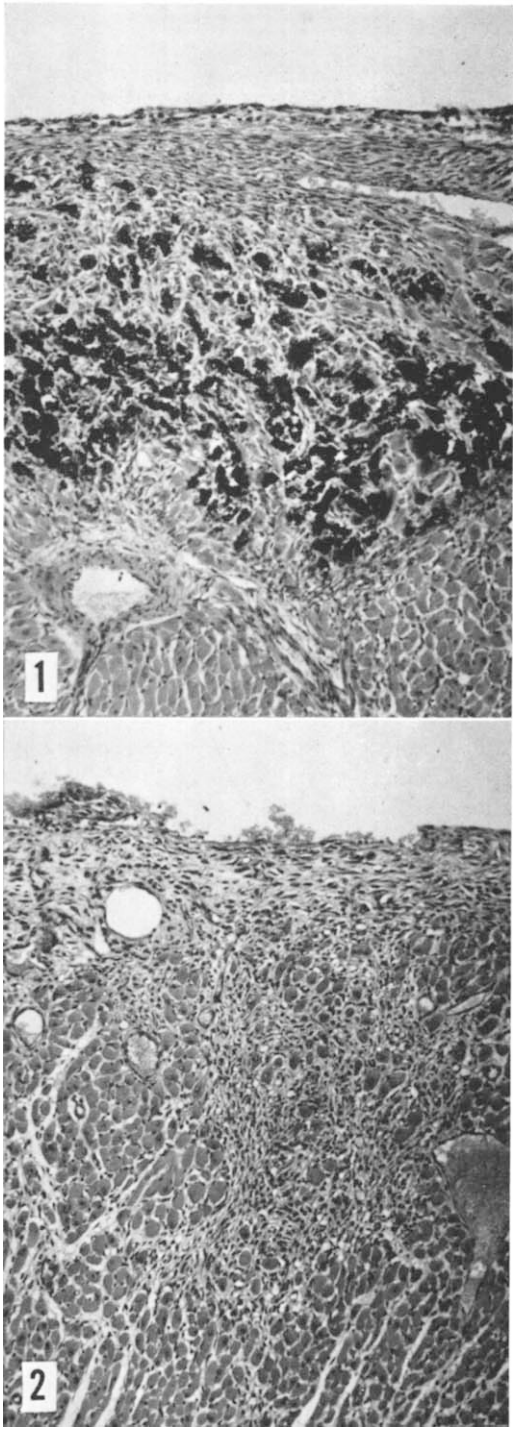

FIG. 1. Microscopic aspects of calcifying cardiopathy produced by ligature of 2 main coronary veins in control rat.

FIG. 2. In a dihydrotachysterol-treated rat, necrosis still develops following obstruction of coronary veins, but secondary calcium deposition in affected areas is prevented (both sections stained with v. Kossa-$AgNO_3$ plus hemat.-phlox.-saffron).

proved to be more or less superficial, sometimes limited to the subepicardial tissue; in other instances they invaded more deeply into the ventricular walls. Calcium deposits always seen in hearts of these otherwise unpretreated rats (Group 1) lie within disintegrated muscle fibers, and in more advanced cases, within the remaining fibrous and granulomatous stroma.

Ligature of the coronary veins resulted in cardiac necroses in 100% of the rats in all 6 groups (Table I). None of the interventions and/or DHT markedly influenced the intensity of myocardial necroses, nor were the morphologic characteristics of the lesions altered by such treatments. On the other hand, subsequent calcification of the damaged myocardial tissue was greatly enhanced by parathyroidectomy (Group 3) and this enhancement was counteracted by simultaneous thyroidectomy (Group 5). The degree of calcification seen in Group 3 as compared with that of Groups 1 and 5 proved to be statistically significant ($P<0.01$); repeated checking of these experiments constantly gave the same or sometimes even larger differences. It is even more interesting that administration of DHT to intact (Group 2), parathyroidectomized (Group 4), or thyroparathyroidectomized (Group 6) rats not only significantly reduced but almost completely prevented the myocardial calcification that otherwise follows coronary-vein obstruction.

Analysis of serum electrolytes indicated that the low calcium and high inorganic phosphorus values seen following parathyroidectomy (Group 3) were not further affected by simultaneous removal of the thyroids (Group 5). It is also obvious that the dose level of DHT used in these studies was not sufficient to produce alterations of normal serum calcium and phosphorus in the intact rats (Group 2), but high enough to restore the electrolyte balance in parathyroidectomized (Group 4) or thyroparathyroidectomized (Group 6) animals.

Discussion. The principal outcome of these studies is that the myocardial calcification

that normally follows coronary vein obstruction is: 1) enhanced by parathyroidectomy, and 2) prevented by adminstritaion of DHT.

Our observation on the aggravating action of parathyroidectomy is at variance with earlier findings indicating that removal of the parathyroids provides protection against development of various calcifying cardiovascular lesions(7,8) and especially of those resulting from impairment of renal excretory functions(9). This contrasting influence of parathyroidectomy on various types of calcifying cardiopathies is particularly interesting; but it remains to be established whether it really indicates alternate mechanisms for the occurrence of calcium phosphate deposits in the affected cardiac tissues. In any case, the observation that simultaneous removal of the thyroids actually counteracts the aggravating influence of parathyroid deficiency cannot be explained simply through the changes seen in electrolyte balance. It is reasonable to assume that this abolishing action is due to a decreased rate in cell metabolism that is known to follow thyroidectomy and it may be that some thyroid principles are necessary for promoting the entry of various ions (calcium, inorganic phosphorus) into the damaged tissue areas.

The observation that the myocardial calcification that normally follows coronary vein ligature can actually be prevented by administration of DHT (a well-known calcifying agent) is as surprising as is the opposite influence exerted by parathyroidectomy. Vitamin D derivatives, including DHT, have been shown not only to act similarly in many respects to parathyroid hormone(10,11) but also to induce severe myocardial and other soft tissue calcification when given in sufficiently large amounts(12). The comparatively low dose of DHT used in the present study was, in fact, able to restore serum calcium and inorganic phosphorus levels to normal in the parathyroid deficient rats, but it had no effect on the serum electrolytes of intact animals, nor did it produce demonstrable soft tissue calcification in either group. DHT was equally effective in parathyroidectomized, thyroparathyroidectomized or intact rats in preventing myocardial calcification, indicating that some direct action on cells is probably involved in this protective mechanism.

Finally, it should be reemphasized that parathyroidectomy and administration of DHT both merely influenced the extent of calcification, not the occurrence and intensity of cardiac necrosis that follows coronary-vein ligature. Thus, a dissociation between calcification and myocardial damage could be demonstrated, which may open new avenues for research on factors involved in soft-tissue calcification.

Summary. Experiments in the rat indicate that ligature of the 2 main coronary veins induces inflammatory and degenerative changes in the subepicardial muscle layers followed by massive calcification. This type of myocardial calcification, unlike the onset of necrosis, is greatly enhanced by parathyroidectomy. The aggravating action of parathyroid deficiency is, however, counteracted by simultaneous removal of the thyroids. On the other hand, administration of dihydrotachysterol (DHT) actually prevents the myocardial calcification in intact, thyroidectomized, or parathyroidectomized rats. DHT merely influences the extent of calcification, not the cardiac necrosis itself. The changes recorded in serum calcium and inorganic phosphorus levels do not help explain the above findings.

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