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THE EFFECT OF PHENYLBUTAZONE UPON DIHYDROTACHYSTEROL OVERDOSAGE IN THE RAT

BY

H. SELYE

From the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

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Experiments on albino rats showed that short-term overdosage with dihydrotachysterol, in amounts causing only traces of soft-tissue calcification, induced a very pronounced calcium deposition in the heart, aorta, and kidney when the animals were simultaneously treated with phenylbutazone. Special attention is called to the fact that, under these experimental conditions, it is possible to obtain consistently a massive calcium deposition in the stroma of the renal papilla similar to that seen in " Randall's plaques " during the development of urolithiasis in man.

It had been shown in earlier publications that corticoid hormones can significantly modify the syndrome of soft-tissue calcification that is induced by vitamin-D derivatives (Selye, 1956, 1957). It seemed of interest to establish whether this singular effect of the adrenocortical hormones could be duplicated with phenylbutazone, since many of the pharmacological actions of the latter resemble those of corticoids.

METHODS

Forty female Sprague-Dawley rats with a mean initial body weight of 101 g. (range, 92 to 110 g.) were subdivided into four equal groups. Treatment with dihydrotachysterol and phenylbutazone was carried out as is shown in Table I. Dihydrotachysterol (" Calcamin," Wander) was given once daily in doses of 100 μg. in 0.4 ml. of sesame oil through a stomach tube. Phenylbutazone (" Butazolidin," Geigy) was administered subcutaneously in two daily injections of 10 or 20 mg. each in 0.2 ml. of water.

Throughout the experiment the animals were fed on " Purina Fox Chow " (Ralston Purina Company Ltd.). After 10 days' treatment all the surviving rats were killed with chloroform; their hearts, aortae, and kidneys were fixed in neutral formalin, embedded in paraffin, and stained with von Kóssa's calcium stain. The intensity of calcification was assessed in an arbitrary scale of 0 to +++ (0=no calcification, += trace of calcification, ++=moderate degree of calcification, +++=intense calcification). The mean of these values as well as the mean final body weight (with its standard error), the % gain in body weight during the period of observation, and the mortality rate are summarized in Table I.

RESULTS

During the last 3 days of the experiment, one rat in Group III and four rats in Group IV died with extensive calcium depositions in the heart, aorta and kidney. It was particularly striking that the renal calcification remained almost exclusively limited to the papilla. It will be remembered that the rat kidney possesses only one papilla.

When survivors were killed on the 10th day, it became evident that, in all animals treated with dihydrotachysterol in combination with phenylbutazone, the renal papillae were chalky white in

TABLE I

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Each group consisted of 10 rats. The arbitrary scale of assessment of the degree of tissue calcification is given in the text.

Group	Phenylbutazone (mg./day)	Dihydrotachysterol (μg./day)	Final Body Weight with S.E. (g.)	Gain in Body Weight (%)	Calcification			Mortality (%)
					Heart	Aorta	Kidney	
I	—	100	83±1·5	−18	0	0 to +	0 to +	0
II	2×20	—	128±4·3	+28	0	0	0	0
III	2×10	100	77±2·2	−23	+ to ++	+++	+++	10
IV	2×20	100	73±2·9	−26	++	+++	+++	40

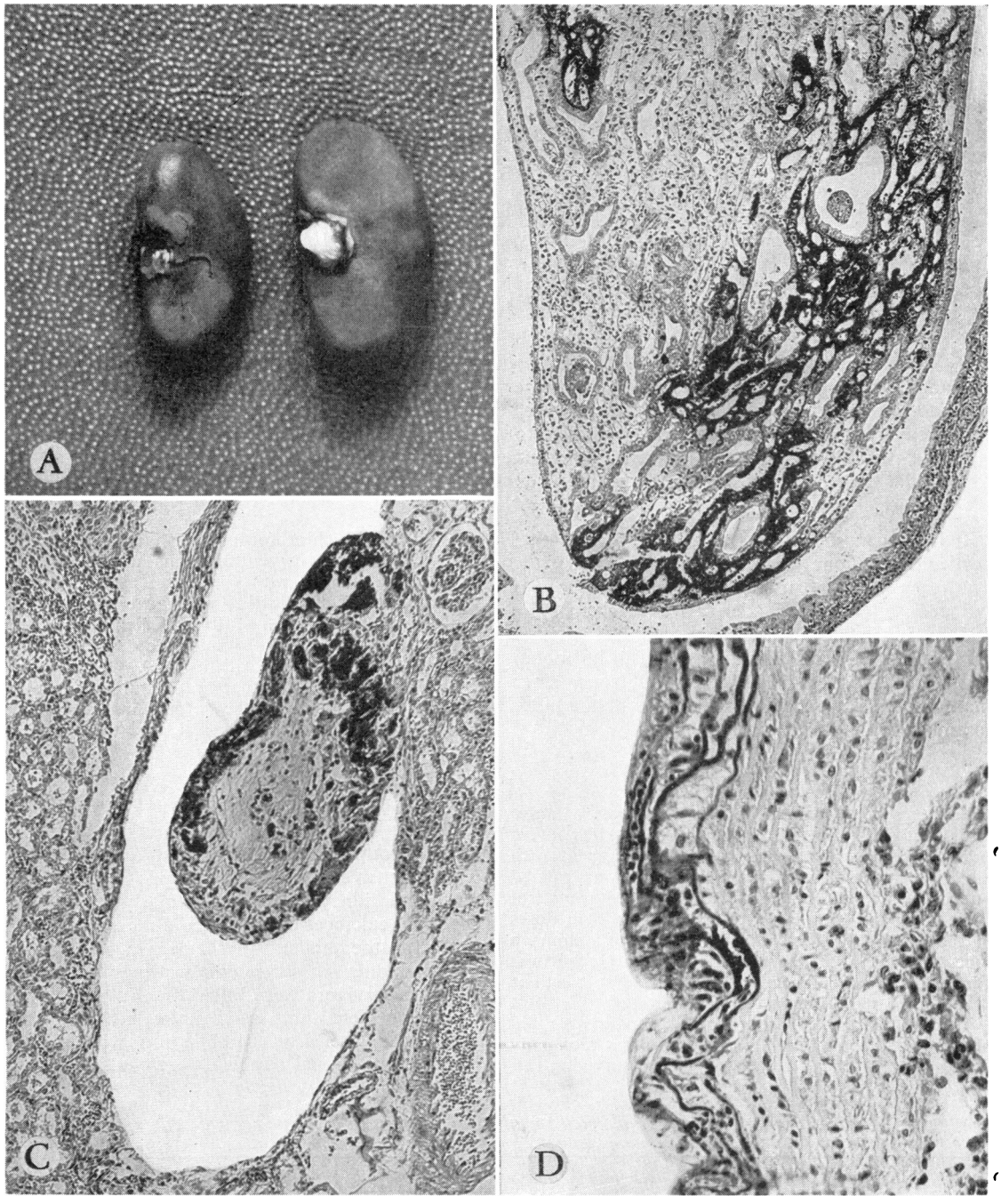

FIG. 1.—A. Kidney of a rat treated with dihydrotachysterol alone (left) and of an animal given dihydrotachysterol and phenylbutazone. Note the calcification (chalky white colour) and great prominence of the renal papilla in the latter kidney. (The light-grey areas in both kidneys are reflections of light unrelated to calcification.) B. Histological aspect of the renal papilla in a rat treated with dihydrotachysterol plus phenylbutazone. The localization of the calcium deposits in the stroma is clearly visible here with von Kóssa stain. ×75 C. Partially calcified "venous cushion," which protrudes from the wall into the lumen of one of the large renal veins near the hilum of the rat kidney shown in B, von Kóssa stain. ×75. D. Wall of the aorta showing the Mönckeberg sclerosis-like calcification in a rat treated with dihydrotachysterol and phenylbutazone, von Kóssa stain. ×320.

colour, and histochemical examination with von Kóssa's stain revealed that this region was deeply impregnated with calcium salts. Calcification was restricted almost wholly to the stroma between the collecting ducts. There was no tendency to form calcified casts such as are observed, for example, in rats which have received a great excess of sodium phosphate in the diet. This incrustation of the renal papillary stroma with calcium is strikingly similar to the initial stages of renal calculus formation, the so-called " Randall's plaque " (Randall, 1937). In two of the animals in Group IV, hard calcified calculi were found loose in the renal pelvis. These stones were presumably caused by the detachment of the calcified area from the papilla and the subsequent deposition of salts upon these foci ; all this is in agreement with Randall's theory.

It is also noteworthy that in Group IV polyp-like protuberances frequently developed in the larger renal veins. These are apparently identical with the " venous cushions " seen in rats simultaneously treated with corticoids and ergocalciferol (Selye, 1956). However, the " venous cushions " in Group IV of the present experiment were invariably calcified whereas those of the rats treated with corticoids and ergocalciferol were not incrustated with calcium salts.

Intense calcification was seen in the aortae and hearts of the rats in Groups III and IV which received phenylbutazone simultaneously with dihydrotachysterol. On the other hand, no calcification was observed in these organs after treatment with phenylbutazone alone, and only traces of calcium were detectable in the aortae and kidneys of the rats treated with dihydrotachysterol alone, in these short-term experiments.

The loss in body weight induced by dihydrotachysterol alone was significantly increased by twice-daily doses of 20 mg. of phenylbutazone. On the other hand, phenylbutazone alone (given at this same dosage) not only failed to cause a loss in body weight but actually permitted a gain of 28% in 10 days, which is approximately normal for rats of this weight range in our colony.

These findings are summarized in Table I and illustrated in Fig. 1.

Discussion

From these observations it is clear that the syndrome of dihydrotachysterol overdosage is significantly modified by concurrent treatment with phenylbutazone. The loss of body weight and the tendency to form calcium deposits in heart, aorta and kidney, during a brief period of treatment with dihydrotachysterol, was greatly enhanced by the concurrent administration of, in themselves, non-toxic doses of phenylbutazone. In all these respects phenylbutazone altered the syndrome of dihydrotachysterol intoxication in much the same way as hydrocortisone acetate acts upon the syndrome of intoxication produced by ergocalciferol in the rat (Selye, 1956). However, combined treatment with hydrocortisone acetate and ergocalciferol results in calcifications, localized predominantly in the aorta, whilst calcification in the renal tissue is less conspicuous and usually limited to the formation of calcified casts (especially in the cortex and the corticomedullary junction line). Combined treatment with phenylbutazone and dihydrotachysterol produces calcium deposition predominantly in the stroma of the renal papillae.

The evidence at our disposal does not warrant the formulation of any hypothesis concerning the mechanism of the interaction between phenylbutazone and dihydrotachysterol. However, it does show that combined treatment with these two substances regularly resulted in the production of changes similar to those in " Randall's plaques " in the lower kidney.

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References

Randall, A. (1937). *Ann. Surg.*, **105**, 1009.
Selye, H. (1956). *Münch. med. Wschr.*, **31**, 1015.
—— (1957). *Acta Endocr., Copenhagen.* In the press.