Sensitization by Corn Oil for the Production of Cardiac Necroses by Various Steroids and Sodium Salts*

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VARIOUS TYPES OF cardiopathies have been produced in the rat by combined treatment with certain steroid derivatives (corticoids, vitamin D, dihydrotachysterol) and sodium salts (especially phosphates, perchlorates and sulfates). One of these is the electrolyte-steroid cardiopathy with necrosis produced by 2α-methyl-9α-chlorocortisol plus Na_2HPO_4. This variant has been carefully examined for its dependence upon dietary factors and was found to be considerably aggravated by the oral administration of various fats (corn oil, peanut oil, olive oil, pork fat, butter and chicken fat) and fatty acids (for example, palmitic, stearic and oleic acid), but not by paraffin oil or glycerin.[1,2]

During the past 10 years, cardiopathy with necrosis elicited by various electrolyte-steroid combinations has been used frequently as a convenient experimental model for the study of "metabolic" or nonocclusive myocardial infarcts, particularly with respect to their histology, histochemistry, electron microscopy, enzyme chemistry and susceptibility to prophylactic treatment. However, the usefulness of this model was limited by the fact that certain strains of rats (such as those of the Holtzman Farms) are singularly resistant to the production of the electrolyte-steroid cardiopathy with necrosis by the usual technics. The insensitive animals usually die of intercurrent infections after long-term, heavy corticoid overdosage before cardiac necroses develop.

Having noted that even resistant strains become very sensitive to electrolyte-steroid necrosis under the influence of orally administered fats, we decided to explore this form of sensitization in greater detail.

MATERIALS AND METHODS

Two hundred female rats of the Holtzman Farms (Madison, Wis.) with an initial body weight of about 100 gm. (range 90 to 110 gm.) were subdivided into 20 equal groups and treated as outlined in Tables I and II. The dosage of the steroids used was 750 μg. for fluorocortisol acetate and 2 mg. for desoxycorticosterone acetate, cortisol acetate, progesterone and methyltestosterone. These amounts were injected in the form of microcrystal suspensions, subcutaneously in 0.2 ml. of water, once daily. The electrolytes employed were Na_2HPO_4, NaH_2PO_4, $NaClO_4$, $NaHCO_3$, $NaHSO_4$ or NaCl, always at the dose of 1 millimol (mM) in 2 ml. of water, administered twice daily by stomach tube. Combined treatment with one of these steroids and sodium salts was given either alone or conjointly with 1 ml. of corn oil by a stomach tube twice daily, as indicated in the tables.

The severity of the cardiac necroses was estimated in terms of an arbitrary scale of 0 = no lesion, 1 = just visible, 2 = pronounced, and 3 = most severe lesions, as previously described.[2] Tables I and II list the means of these readings (with their standard errors) including those of animals that died during

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the experiment and those of the remaining rats, which were killed with chloroform after 12 days. The macroscopic findings were confirmed by histologic observation of paraffin-embedded sections stained with the periodic acid-Schiff technic.

Results

Table I deals with the effect of corn oil upon the action of various steroids administered in combination with Na_2HPO_4; Table II summarizes the findings obtained by treatment with the most active of these corticoids (fluorocortisol) in combination with various sodium salts.

As shown by Table I, corn oil supplements greatly increased not only the cardiac necrosis (Fig. 1) and mortality produced by fluorocortisol, but also the characteristic corticomedullary nephrocalcinosis that complicates electrolyte-steroid cardiopathy especially when sodium is administered in the form of phosphates.

It had been shown previously that, for the optimal production of an electrolyte-steroid cardiopathy, it is essential to administer steroids possessing both gluco- and mineralocorticoid effects (e.g., fluorocortisol); virtually pure mineralocorticoids (e.g., desoxycorticosterone) or glucocorticoids (e.g., cortisol) are almost completely devoid of cardiotoxic activity under ordinary conditions.[2] Yet, in the present series, when combined with corn oil, even desoxycorticosterone produced consider-

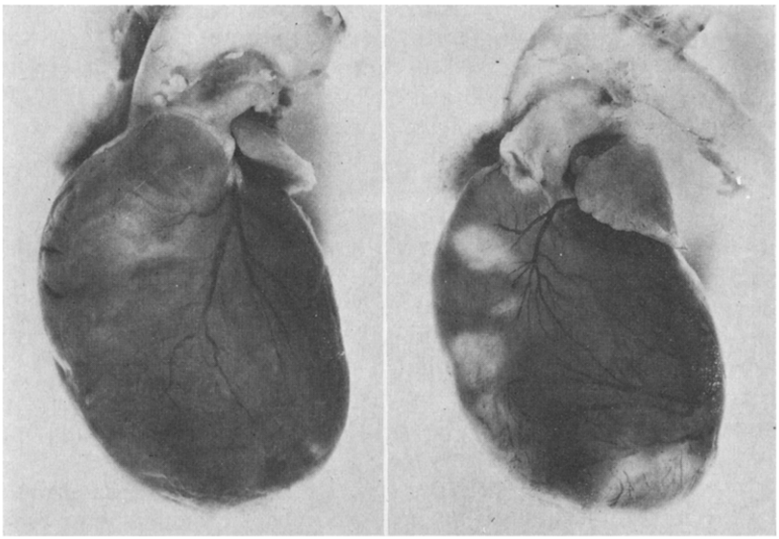

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Figure 1. *Sensitization by corn oil for the production of experimental cardiac necroses.* Both rats were treated with fluorocortisol and Na_2HPO_4. **A,** normal heart of otherwise untreated surviving rat. **B,** several necroses in the wall of the right ventricle and the apex of the heart in a rat which died after the same treatment plus corn oil supplements.

Table I. Effect of Corn Oil upon Cardiopathy with Necrosis Produced by Various Steroids plus Na_2HPO_4

Group	Treatment*	Cardiac Necrosis (scale: 0-3)	Nephrocalcinosis (scale: 0-3)	Mortality (%)
1	Fluorocortisol	0	0.1 ± 0.1	0
2	Fluorocortisol + oil	2.3 ± 0.4	1.8 ± 0.2	80
3	Desoxycorticosterone	0	0.6 ± 0.1	0
4	Desoxycorticosterone + oil	1.5 ± 0.4	1.8 ± 0.3	60
5	Cortisol	0	0	0
6	Cortisol + oil	0	0.4 ± 0.3	0
7	Progesterone	0	0	0
8	Progesterone + oil	0	0.6 ± 0.2	0
9	Methyltestosterone	0	0	0
10	Methyltestosterone + oil	0	0.3 ± 0.1	0

* All rats also received Na_2HPO_4 (see text).

able cardiac necrosis, nephrocalcinosis and mortality, whereas cortisol, progesterone and methyltestosterone proved to be inactive in this respect despite the oil supplements. The traces of nephrocalcinosis noted in some of the oil-treated rats among these groups are of doubtful significance since phosphates alone can occasionally cause renal calcification, although usually only after more prolonged treatment.

Under the same experimental conditions, numerous control rats treated with croton oil alone never showed cardiac necrosis or nephrocalcinosis, although slight lipid infiltration of the tubular epithelium was sometimes detectable.

Table II shows that, without oil supplements, fluorocortisol in combination with various sodium salts failed to produce electrolyte-steroid cardiopathy with necrosis, nephrocalcinosis, or mortality under our experimental conditions. Only $NaClO_4$ caused a 50 per cent mortality, but this was apparently not due to heart failure since only 1 rat of this group showed minor cardiac lesions at autopsy. It is known that $NaClO_4$ produces often fatal convulsions in the rat even under conditions not conducive to myocardial damage.[2]

On the other hand, in the rats also receiving oil, severe cardiac necroses and a high mortality were elicited by fluorocortisol plus NaH_2PO_4, $NaClO_4$ and $NaHSO_4$. $NaHCO_3$ was somewhat less effective in this respect, whereas NaCl caused no cardiac necrosis or nephrocalcinosis even in combination with oil, although it did elicit some mortality.

It should be mentioned incidentally that, in addition to the cardiac necroses, the rats receiving fluorocortisol, $NaClO_4$ plus corn oil exhibited extensive focal hepatic necroses. No such lesions were found in the livers of the animals treated only with fluorocortisol and $NaClO_4$.

Discussion

These experiments clearly show that oral administration of corn oil supplements greatly increases the sensitivity of the rat to the production of electrolyte-steroid cardiopathy with necrosis by various means. Nothing is known about the mechanism through which fats exert this cardiotoxic effect; however, our experiments suggest that they do not act merely by enhancing the toxicity of the sodium ion. It will be noted that the same amount of corn oil augmented the toxicity of 1 mEq. of Na given as NaH_2PO_4 (Table II, Group 2) to approximately as much as that of 2 mEq. administered as Na_2HPO_4 (Table I, Group 2), whereas it caused no cardiac necroses in combination with 1 mEq. of Na supplied as NaCl (Table II, Group 10).

With regard to the part played by the steroid component of the treatments conducive to cardiopathy with necrosis, our findings confirm the previously made observation that optimal sensitization is obtained only by compounds possessing both gluco- and mineralocorticoid activity (e.g., fluorocortisol).[2] These steroids can produce cardiac necroses in combination with appropriate sodium salts even without oil supplements, at least in susceptible strains of rats at high dose levels. However, when given with oil, a virtually pure mineralocorticoid such as desoxycorticosterone is also effective in this respect, whereas cortisol, an almost exclusively glucocorticoid compound,

Table II. Effect of Corn Oil upon Cardiopathy with Necrosis Produced by Fluorocortisol plus Various Sodium Salts

Group	Treatment*	Cardiac Necrosis (scale: 0–3)	Nephrocalcinosis (scale: 0–3)	Mortality (%)
1	NaH_2PO_4	0	0	10
2	NaH_2PO_4 + oil	2.3 ± 0.4	1.9 ± 0.3	90
3	$NaClO_4$	0.2 ± 0.2	0	50
4	$NaClO_4$ + oil	2.8 ± 0.2	0.1 ± 0.1	100
5	$NaHCO_3$	0	0	0
6	$NaHCO_3$ + oil	0.8 ± 0.4	0	30
7	$NaHSO_4$	0	0	0
8	$NaHSO_4$ + oil	2.9 ± 0.1	0.4 ± 0.2	80
9	NaCl	0	0	0
10	NaCl + oil	0	0	30

* All rats also received fluorocortisol (see text).

is ineffective even in combination with oil.

We may conclude that the production of electrolyte-steroid cardiopathy with necrosis is greatly facilitated by the oral administration of corn oil in addition to the standard treatments. This technic provides a convenient test object for the study of metabolic cardiac necroses and particularly for the analysis of the part played by fats in their pathogenesis.

Summary

In the rat, the production of extensive and usually fatal myocardial necroses by fluorocortisol or desoxycorticosterone in combination with Na_2HPO_4 is greatly accelerated and aggravated by the oral administration of corn oil. Cortisol, progesterone and methyltestosterone do not produce myocardial necroses under these conditions even if, in addition to Na_2HPO_4, corn oil is given.

Not all sodium salts are equally potent in producing myocardial necroses when administered in combination with fluorocortisol and fat supplements. In this respect, NaH_2PO_4, Na_2HPO_4, $NaClO_4$ and $NaHSO_4$ are most effective, $NaHCO_3$ is somewhat less active, whereas NaCl is inactive under otherwise comparable conditions.

References

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