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Effects of Two Erythrophleum Alkaloids on Potassium Transfer in Human Erythrocytes.* (27533)

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Alkaloids isolated from the bark of *Erythrophleum guineense* have been known since 1875(1). Maling and Krayer(2) found that the erythrophleum alkaloids cassaine, norcassaidine, erythrophleine, and coumingine produced effects in the dog heart-lung preparation which were similar in some respects (increased contractile force and production of irregularities) to those produced by the cardiac glycosides. Further investigations (for a summary, see 3) have supported these conclusions. The chemical relationships of cassaine and coumingine to cassaic acid have been known for some time (cf. 2); recently the structure of cassaic acid has been demonstrated by Turner *et al.*(4). These structures are shown in Fig. 1. In contrast to the cardiac glycosides, the erythrophleum alkaloids have no cyclopentane ring fused to the phenanthrene nucleus, nor do they have a lactone ring in the molecule. Two other features thought necessary for glycoside-like effects (5), a C-14 –OH group and a cis-fusion of the A and B rings, are also lacking in the erythrophleum alkaloids. Since there is evidence indicating that the latter have cardiac effects like those of the glycosides, the present experiments were done to determine whether the 2 kinds of compounds also share a non-cardiac effect previously unique to glycosides and their lactones, *i.e.,* inhibition of active K accumulation by human erythrocytes (RBC).

Methods. Blood was obtained and treated as previously described(6). Cassaine sulfate and coumingine hydrochloride were dissolved in K-free 0.9% NaCl, and added to 4.2-4.9 ml of blood in volumes up to 0.1 ml. Control flasks received an equal amount of 0.9% NaCl. $K^{42}Cl$ was added in concentrations less than 0.05 meq/l blood; radioactivity was such as to produce counting rates of 1-3 $\times 10^4$ cpm per ml of plasma. Incubation was for 3 hours at 37°. Before and after incubation, measurements were made of whole blood and plasma K^{42} in the well of a scintillation counter, and total K in a Baird KY-1 flame photometer. Micro-hematocrits were done in duplicate. Changes of K from plasma to cell over a 3-hour period were estimated from calculations of specific activities and measurements of K^{42}.

Results. In 9 experiments, the initial concentration of plasma K was 5.24 ± 0.28 (S. E.M.) μeq/ml plasma. The mean control rate of K transfer into the RBC during 3 hours of incubation was 4.98 ± 0.22 μeq K/ml RBC/3 hours; this K exchange rate is similar to those reported previously (cf. 6).

The data obtained with the alkaloids are given in Fig. 2. Both cassaine and coumingine could inhibit active K accumulation by

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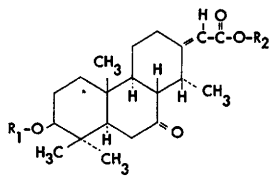


Cassaic acid: $R_1 = R_2 = -H$

Cassaine: $R_1 = -H$; $R_2 = -CH_2CH_2N(CH_3)_2$

Coumingine: $R_1 = (CH_3)_2 = C - CH_2 - C$ (OH, O)

$R_2 = -CH_2CH_2N(CH_3)_2$

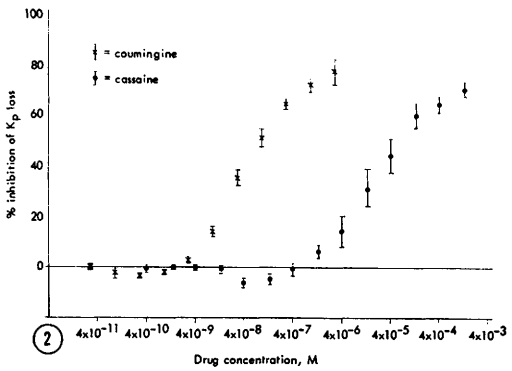
① ② 

FIG. 1. Structure of cassaic acid, cassaine, and coumingine. Structure of the acid is from Turner *et al.*(4).

FIG. 2. Concentration-effect curves: effect of cassaine and of coumingine on transfer of potassium from plasma to cells of fresh human blood. Bars represent $\pm$ 1 s.e.m. Slopes of the 2 curves are the same. Each point represents the mean of 3 to 5 experiments.

the RBC. The I_{50} (concentration producing 50% inhibition) was 10^{-7} M for coumingine, and 5×10^{-5} M for cassaine. The potency of coumingine is close to that of ouabain(6), the most potent cardiac glycoside. The inhibition of K transfer by the erythrophleum alkaloids was similar to that by cardiac glycosides in several respects. It was unaccompanied by any detectable hemolysis. It was incomplete even at high drug concentrations; the concentration-effect curve leveled off below 100% inhibition(7). It was decreased by high levels of plasma K; this phenomenon was not quantified nor studied systematically, but in 2 experiments an increase of $(K)_p$ to 30 μeq/ml plasma reduced the effectiveness of cassaine to about the same extent as did a similar change of $(K)_p$ to the effectiveness of ouabain(8).

Finally, the effects of the 2 classes of drugs at low concentrations were similar. When cassaine or coumingine was present in concentrations of 10^{-3} to $10^{-2} \times I_{50}$, K transfer was slightly increased relative to controls. This increase was not large, about 5% greater than the control rate, but it was noted in all experiments. The same phenomenon has been observed for ouabain (Kahn, J. B., unpublished). Repke(9) has observed activation of a Na + K-activated membrane ATPase by low concentrations of cardiac glycosides. This enzyme has been implicated in processes of active transfer of K across the erythrocyte membrane(10,11). Hence it seems likely that erythrophleum alkaloids, like cardiac glycosides, can increase the activity of the transfer ATPase in low concentrations and inhibit it in high concentrations.

Discussion. In all effects here studied and in the cardiac effects previously investigated, the erythrophleum alkaloids are similar to the cardiac glycosides. Yet the structures have little in common. The minimal structural requirements for cardiotonic activity of the glycosides are generally thought to be: a cyclopentanophenanthrene nucleus with A/B cis-, B/C trans-, and C/D cis-fusion of the rings, a C-14 –OH group, and a saturated or unsaturated γ- or δ-lactone ring in the β-configuration on C-17. The erythrophleum alkaloids satisfy none of these requirements, and still are biologically equivalent to the structures which do fulfill them, the cardiac glycosides. It would appear that the minimal requirements for glycoside-like activity must be changed. When 3-dimensional models of both classes of compounds were made, a feature which they had in common was that the distance between the C-3 oxygen and the carbonyl oxygen was 11.5 Å in both cases. Other than this, no striking similarities in the models were apparent. If the minimal requirement for cardiotonic activity is in fact a hydroxyl oxygen and a carbonyl oxygen separated by 11.5 Å, the structural basis for activity would be very much simplified, and our views of the structure-activity relations of cardiotonic compounds might be usefully revised.

Summary. The erythrophleum alkaloids, cassaine and coumingine, affect active trans-

port of potassium by human erythrocytes as do the cardiac glycosides. At low concentrations both groups of compounds slightly increase K transfer from plasma to cells; higher concentrations inhibit the process. These effects probably reflect activation and inhibition of a membrane ATPase which others have implicated in the active movements of cations in erythrocytes. Erythrophleum alkaloids and cardiac glycosides also share similar effects on mammalian hearts. In view of the structural differences between the 2 classes of compounds, and their biological similarities in 2 kinds of tissue, it is suggested that previous views on minimal structural requirements for digitalis-like activity in hearts and erythrocytes be revised.

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Lesser Effects of Endotoxin on Intestinal Weight in Young *versus* Older Dogs.* (27534)

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Response to intravenous administration of *E. coli* endotoxin in adult dogs has been characterized, in part, by an initial drop in systemic pressure, and an increase in portal venous pressure and intestinal weight(1). Weil and Spink(2) observed that shock could not be uniformly produced in immature animals. Zahl *et al.*(3) suggested that younger mice were more resistant to a lethal dose of endotoxin. Young rabbits were capable of withstanding a 50 times greater dose of endotoxin than adults as judged by 18 hours survival (4). In rabbits the vascular effects of endotoxin have been investigated in reference to the Schwartzman reaction(5). Others(6) have found an increased susceptibility of rabbits to the Schwartzman phenomenon with age.

Alterations in intestinal weight in adult dogs during the first 10 minutes following endotoxin administration have been compared with similar changes following mechanical increase in portal pressure for 10 minutes(7). The purpose of this investigation is to compare intestinal weight responses of mature and immature dogs during the period of portal hypertension and later stages of endotoxin shock.

Methods. Experiments were preformed on 5 adult and 6 young dogs. The teeth were used as criteria for establishing age. All

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