SMOOTH MUSCLE CONTRACTOR ACTIVITY OF COTTON DUST AQUEOUS EXTRACT *IN VITRO.* III - FRACTIONATION SUGGESTS THAT DIFFERENT COMPONENTS ARE RESPONSIBLE FOR THE CONTRACTIONS INDUCED IN THE RAT STOMACH FUNDUS AND THE GUINEA PIG TRACHEA.

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Abstract

A number of studies have shown that aqueous extracts of cotton dust cause contraction of a variety of smooth muscle preparations. Accordingly, it has been postulated that there is a "smooth muscle contractor agent" in cotton dust. Using solvent extraction techniques, this study provides evidence that there are at least two rat fundus contractor agents neither of which fits the profile of activity observed in the isotonic guinea pig isolated trachea preparation.

Introduction to Part III

Fractionation Suggests that Different Components are Responsible for the Contractions Induced in the Rat Stomach Fundus and the Guinea Pig Trachea.

In exposed mill workers, inhaled washed cotton does not cause the same degree of reduction in lung function as unwashed cotton [1]. Acetone extracts and chloroform:methanol (2:1) extracts of cotton dust aqueous extract elicited no contractile activity in the guinea pig ileum or guinea pig trachea and only a trace of activity the rat fundus activity [2]. These and other studies (see part V of this series of reports) suggest cotton dust constituents active on the rat fundus are highly polar.

In our studies, ether extracts of CDE were found not to elicit responses in smooth muscle tissues. Ether is a "moderately polar" solvent with a solubility parameter (SP) of 7.4H. Water has an SP of 23.4H and is highly polar. Water and ether are immiscible. (1,4-)Dioxan is a cyclic diether. It, too, is a "moderately polar" solvent and has an SP of 10.0H. It has the advantage of being completely miscible in all proportions with water and "non-polar" hydrocarbons such as n-heptane, allowing a spectrum of solubility parameters and polarities to be obtained with just three solvents if required. In our preliminary studies, pure dioxan extracts of CDE did not elicit responses in the modified rat fundus or the guinea pig perfused lung preparations.

Pyridine is also completely miscible with water. However, its dipole interactions are much greater than dioxan. Pyridine extracts of CDE did not produce responses in the perfused lung preparation and although, from a theoretical standpoint, pyridine would be a more suitable tool for solvent extraction, it was rejected on grounds of toxicity. Therefore, dioxan was employed as a convenient alternative.

Using mixtures of dioxan and water, a series of solvents was developed for graded separation of CDE on the basis of polarity. These fractions were then employed in a comparative study of their effects on the isotonic rat stomach fundus strip and the isotonic guinea pig trachea preparation (GPT).

Materials and Methods

A list of the materials used can be found in Table A of part I of this series of reports.

The tissues were prepared as described in part I and part II of these series of reports for the rat stomach fundus and the immersed guinea pig trachea preparation respectively, carbachol being the internal standard reference.

Extraction Procedure

The extraction procedure is shown in figure 1. In the final stage, the 95% aqueous dioxan was removed by blow drying under low pressure, followed by reconstitution in water to the required concentration (calculated as whole CDE concentration equivalent).

The Fractions

The fractions were labelled in rank order of increasing hydrophilicity; P1 (precipitate of procedure 1), P2 (precipitate of procedure 2), P3 (precipitate of procedure 3), and S3 (supernatant of procedure 3).

Cumulative and Relative Responses

The effect of adding 5μg/ml CDE-equivalent doses of the fractions cumulatively was examined in both GPT and the rat fundus.

The relative potency of responses was obtained by administering an unfractionated CDE dose-equivalent to each tissue. For example, if CDE (100mg) were fractionated, each fraction would then be dried and re-suspended in water (0.5ml), and applied to the bath. The response would be compared with that to 0.5ml of a 200mg/ml solution of fresh, unfractionated, CDE.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:346-349 (1996)
National Cotton Council, Memphis TN

Results

Table 1 - The percentage mass and colour of the fractions.

Fraction	% mass fraction of Whole CDE	Colour
P1	10.5(±2.3)	grey
P2	39.2(±4.5)	grey
P3	38.6(±3.7)	red *
S3	11.7(±3.7)	yellow

* The characteristic odour of CDE appeared in this fraction.

The Fractions' Dose-Response Curves in the Rat Fundus (fig. 2)

The results are summarised in table 2.

Fraction S3 had an ED_{50} of 11.3(±3.2)μg/ml (n=8). This was not statistically different from fresh CDE (ED_{50} = 10(±2.5)μg/ml). Fraction P3 with an ED_{50} of 29.3μg/ml was 2.6 times less potent than S3. Surprisingly, the decreasing order of potency did not follow the same pattern as decreasing hydrophilicity, so that P1, with an ED_{50} of 57(±15)μg/ml was 3.3 times more potent than P2 with an ED_{50} of 185(±53)μg/ml.

Table 2 - The Potency of the Fractions on the Rat Fundus.

Fraction	ED_{50} μg/ml of whole CDE equivalent	SEM	N
P1	**57.0**	**15**	**7**
P2	**185**	**53**	**7**
P3	**29.3**	**7.8**	**7**
S3	**11.3**	**3.2**	**8**
Whole CDE	**10.0**	**2.5**	**34**

Effects of the Fractions in GPT (fig. 4)

Individual fractions produced comparable responses, each being approximately 20% of that expected from an equivalent dose (5mg/ml) of whole CDE. Based on the CDE D-R curve in GPT (fig. 3), such a response would be expected from a 50μg/ml dose of whole CDE i.e. a one hundred-fold drop in potency. However, the cumulative effect of the four fractions did not show a significant drop in overall response (84(±9.5)% control, $p>0.18$).

Discussion

From data presented in part I and II of these series of reports, the first comparison to be made between CDE effects in the rat fundus and in GPT is that CDE is 30 times more potent in RF than in GPT (compare ED_{50}'s). Following fractionation, almost all of the rat fundus activity was present in the S3 fraction. Nevertheless, if the component appearing in the S3 fraction were the only rat fundus contractor agent, then progressive increase in solvent hydrophobicity would be expected to result in successive decreases in compound solubility. However, construction of D-R curves and evaluation of the ED_{50}'s of the individual fractions revealed a reversal of the order of potency of the fractions with respect to hydrophilicity; P1 being more potent than P2, despite P1's dry mass being more than 3 times P2's. Two explanations may be proposed for this observation: There may be a rat fundus relaxant component in P2 or there may be a second, minor, rat fundus contractor component in P1.

Unlike in the rat fundus, the distribution of the contractor agent(s) in GPT was approximately equal in all fractions. In GPT, each fraction appeared to elicit 20% of the whole CDE response. This is equivalent to a two-log drop in potency. However, when the fractions were added to the bath in succession, the cumulative effect was almost the same as whole CDE. One explanation for this discrepancy may be that there is more than one GPT contractor agent in CDE, whose effects may be synergistic. Such a hypothesis is supported by the finding that there are at least two pharmacological mechanisms by which CDE results in a contraction of GPT (see part II of this series of reports).

For the present, it can be concluded that there is, probably, only one major plus at least one minor rat fundus contractile mediators. This is supported by our findings that there is more than one pharmacological mechanism for the CDE-induced contraction of the rat fundus (see part I of this series of reports). In the present work, it has been found that, the solubility-activity profile of the rat fundus and the GPT contractor agents do not match. The evidence also suggests that there is, probably, more than one GPT contractor agent in CDE.

Previous work in this department [3] using 67% aqueous acetone has demonstrated the same difference in hydrophilicity of contractor agents with respect to different tissues: all of the rat fundus contractor activity was found to be present in the 67% aqueous acetone soluble fraction, whilst this fraction and its respective precipitate were found to contain approximately equivalent bronchoconstrictor activity in the guinea pig perfused lung. This suggests a similarity between the mechanism of action of CDE in the guinea pig trachea and the perfused lung in this respect. Further similarity between the responses of these tissues to CDE is indicated by the results of the superfused tracheal preparation (see Part II of these series) which suggest a mediator release mechanism in GPT. This is further supported by the comparative response profile of GPT and the rat fundus. In the latter, where the evidence suggests a direct receptor acting component, the observed responses were rapid to reach a plateau and quick to return to baseline following washing. In both GPT and the perfused lung, CDE-induced responses are very slow to reach a plateau and slow to return to baseline. Both histamine [4] and arachidonic acid metabolite [5] release from the guinea pig perfused lung have been reported. Our studies have shown that substances capable of modifying the action of these mediators reduce CDE-induced responses in GPT (see part II of these series of reports).

Given that byssinosis is a complex syndrome, it is not surprising to find many, potentially contributory,

pharmacologically active agents and mechanisms which vary, depending on tissue type. Diverse *in vitro* studies of the agents and mechanisms of airborne dust components will, hopefully, provide more informed and directed *in vivo* attempts to understand and prevent byssinosis.

References

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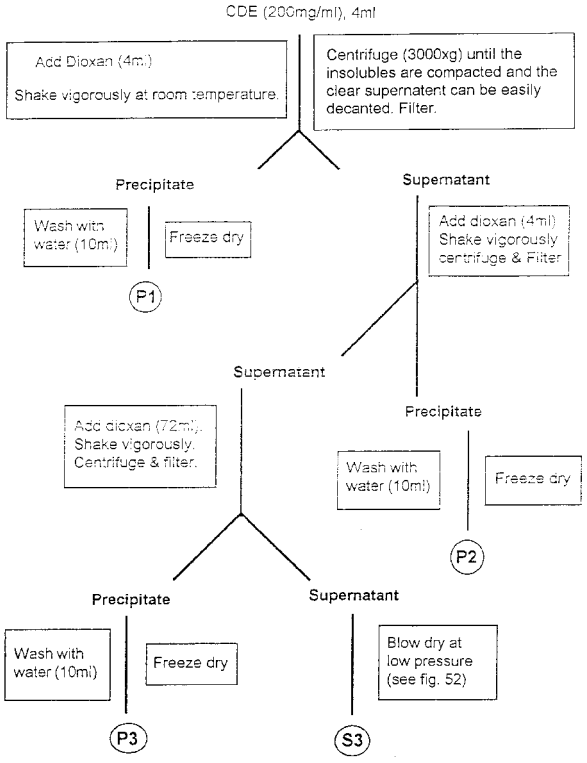

Figure 1. Procedure for the Separation of Aqueous Dioxan Fractions.

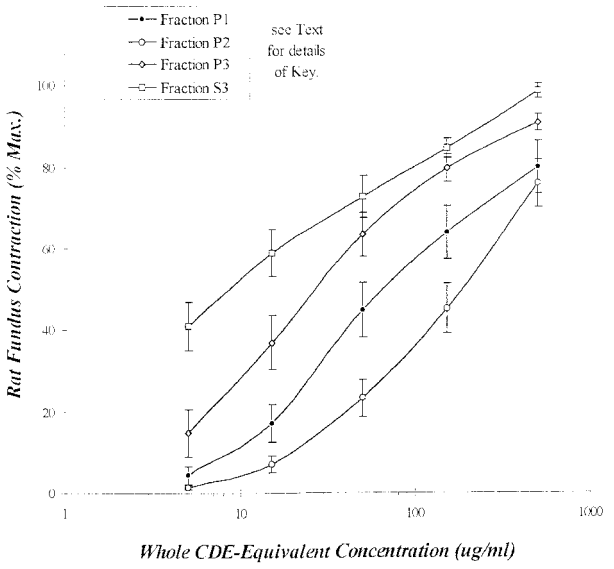

Figure 2. Dose-Response Curves of the Aqueous Dioxan Fractions of CDE in the Modified Rat Stomach Fundus Preparation.

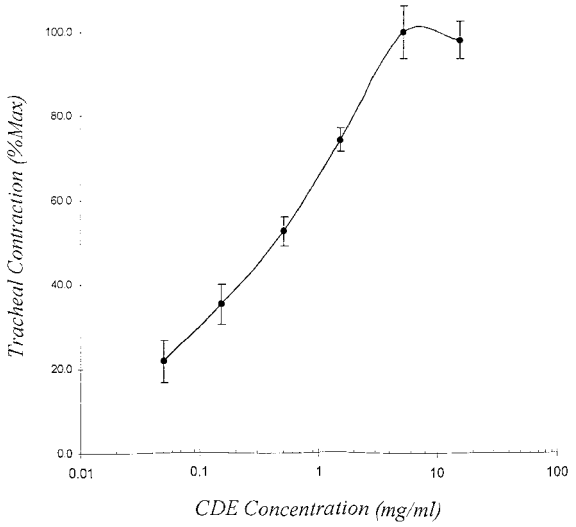

Figure 3. The CDE Concentration-Response Relationship in the Isotonic Guinea Pig Trachea as a Percentage of Its Maximum Response.

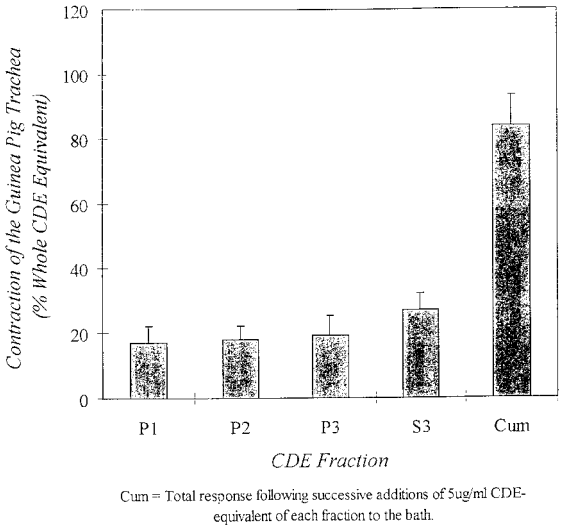

Figure 4. Responses of the Guinea Pig Trachea (Isotonic) to the Individual Fractions of CDE Expressed as the Same Concentration Equivalent (5mg/ml) of Whole CDE.