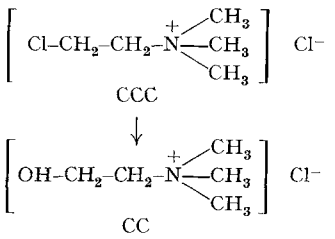
Some Factors Affect the Degradation of (2-Chloroethyl) Trimethylammonium Chloride by Wheat Plant Extracts

Treating wheat plants with (2-chloroethyl) trimethylammonium chloride (CCC) leads to plants with shorter stems, darker green leaves and makes them more resistant against lodging[1,2]. CCC is a choline ester and is not yet known to be a natural compound in plants, although many other choline esters occur naturally in plants[3]. CCC was found to be degraded quickly by soil microorganisms[4,5]. It was reported in a previous study that CCC was degraded in vivo in plants and grains of wheat. In in vitro experiments, CCC could be degraded by extracts of wheat and other plants to choline chloride (CC)[6]. In barley and chrysanthemum shoots, CCC was found to be converted to CC[7], while others found that this compound undergoes very little or no metabolism in wheat plants[8]. In this study, investigations were made on the in vitro degradation of CCC by wheat plant extracts and the factors influencing this degradation.



Materials and methods. Young wheat plants (15–20 days old) were ground with purified sea-sand in a cold mortar. The homogenate was diluted with distilled water, filtered and the filtrate was also diluted with distilled water to give a final concentration of weight of plant material: total volume of 1:5.

If not otherwise indicated, each 25 ml incubated measuring flask contained 5 ml extract, 5 ml 10^{-3} CCC solution, 5 ml acetate buffer at pH 6 and made up to mark with H_2O. Incubation temperature was 30 °C. After 24 h, 5 ml were taken for the determination of CCC and CC, using a thin layer chromatography method[9].

Results and discussion. (1) *Influence of pH.* (a) Preincubation treatment. The plant extract was preincubated for 30 min at pH values ranging from 2–12. After this treatment pH was adjusted to 6.0. Then CCC was added and the determination of CCC degradation was carried out as above. Figure 1 shows that the pretreatment with extremely high or low pH values influenced the ability of plant extract to convert CCC to CC negatively. Only at pH 6–9 its ability was not affected. (b) Incubation at different pH values. Incubation of extract + CCC was done at pH values, that ranged from 2–7 using phosphate acetic acid buffer. No incubation at pH over 7 was carried because CCC is decomposed in the alkaline medium. Figure 2 shows that the ability of the extract to degrade CCC was increased by increasing the pH of the medium and reached a maximum at pH 6.0.

(2) *Influence of temperature.* (a) Preincubation treatment. Heating the plant extract in a water-bath at different temperatures for 10 min (40–90 °C) before incubation, then cooling it to room temperature and then incubating it, did not affect its degradation ability. However, boiling it for different periods ($^1/_2$–5 h) caused an inactivation. (b) Incubation at different temperatures. Increasing the incubation temperature from 10–40 °C caused a remarkable increase in the ability of the extract to convert CCC to CC (Figure 3).

(3) *Effect of dialysis.* By dialysing the extract for different periods in H_2O at 5 °C in a cellulose bag, it was found that it loses its ability to degrade CCC gradually with increased dialyses. After a period of 24 h, the extract was not able to convert CCC to CC. Also the dialyses water alone could not convert CCC to CC. When com-

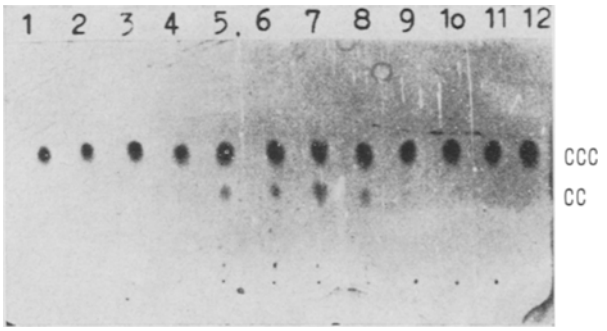


Fig. 1. Influence of preincubation of plant extract with different pH values. 1–11, pH 2–12; 12, CCC without plant extract.

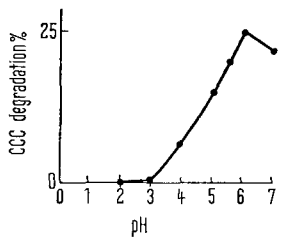


Fig. 2. Influence of the incubation pH on the degradation of CCC.

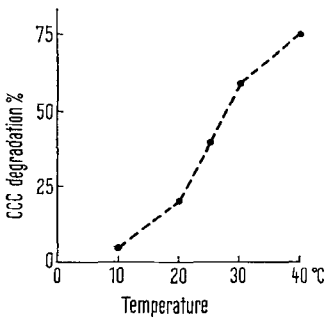


Fig. 3. Influence of incubation temperature on the degradation of CCC.

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bining them again, they could, but not with the same intensity as when the undialysed extract was used (Figure 4).

(4) *Protein precipitation.* Protein was precipitated from the plant extract using $ZnCl_2$; it was then filtered and used to determine its ability to convert CCC to CC. The extract after protein precipitation was not able to convert CCC to CC (Figure 4). From the numerous experiments carried out, it was observed that: (a) The conversion of CCC to CC seems to be a balanced reaction. (b) Addition of toluene (1 ml/incubation flask) or chloramphenicol (5 mg/incubation flask) to the extract led to a small reduction in its ability to convert CCC to CC.

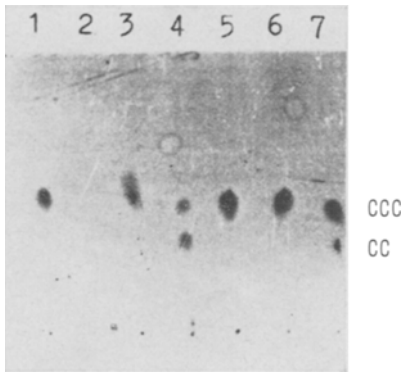

Fig. 4. Effect of different treatments of extract on the degradation of CCC. 1, CCC without extract; 2, extract without CCC; 3, extract after protein precipitation ($ZnCl_2$) + CCC; 4, untreated extract + CCC; 5, dialyses water + CCC; 6, dialysed extract + CCC; 7, dialysed water + dialysed extract + CCC. All components of extract used were equal.

Wheat plant extracts contain a system which can convert CCC to CC in vitro. This system is affected by pH and seems to be thermostable. Dialyses showed that this system has at least 2 components, one of them is precipitated with $ZnCl_2$ (protein), while the other is dialysable; only in the presence of both, the extract can convert CCC to CC. This system might be enzymatic (cholinesterase). Nevertheless, more detailed studies are needed.

Zusammenfassung. Extrakte aus Weizenpflanzen enthalten ein System, welches Chlorocholinchlorid zu Cholinchlorid umwandelt. Dieses System wird durch pH, aber nicht durch die Temperatur beeinflusst und hat zwei Komponenten; die erste wird durch $ZnCl_2$ gefällt, und die andere ist dialysierbar.

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Correlation Between K_1 and K_2 in the Fractional Clearance of Bromosulphthalein in Cattle and Sheep Suffering from Different Hepatotoxic Diseases

Bromsulphthalein (BSP) fractional clearance proposed by Ingelfinger et al.[1] and Lewis[2,3] has been studied in man[1–6], dog[7], turkey[8], horse[9,10], sheep[11–16] and cattle[17–22].

Attention has been drawn to the fact that the excretion pattern observed in cattle and sheep differs from other species in which a biphasic pattern of excretion is generally observed in the normal animal. In the normal ruminant however a single straight line is generally obtained and a biphasic pattern is suggestive of liver dysfunction[21,22].

Wirtz et al.[23] have suggested that the process of hepatic excretion of BSP and similar pigments involves a dual mechanism; (a) prompt removal of the pigment from the blood and its temporary storage in the liver cells and (b) its gradual subsequent transfer from these cells into the lumen of the bile canaliculi. They concluded that the delay of transfer of BSP from the hepatic cells into the lumen of the bile canaliculi constitutes the first manifestation of impairment of the capacity of the liver for excreting BSP and similar substances, and removal of dye from the blood is relatively unaffected.

The values for K_1 (K where there is a single phase curve) are determined by the mechanism(s) which control uptake of dye by the liver cell i.e. the elimination of BSP from the circulation. Other steps in the metabolism of BSP; conjugation[24,25], storage, and excretion to the bile[26], can only affect the value of K_1 when they are unable to keep pace with the uptake of dye. During the initial period when the latter systems are not saturated with BSP, a uniform rate of excretion is observed (K_1). A stage is reached when the uptake of dye from the blood is limited by the rate at which BSP can be passed on to

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