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

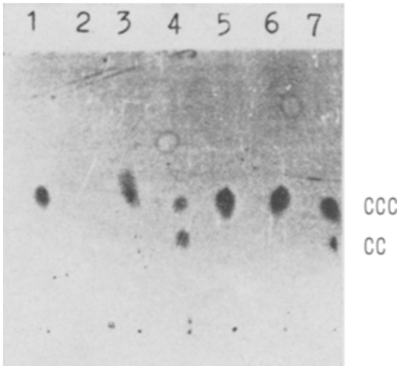

Fig. 4. Effect of different treatments of extract on the degradation of CCC. 1, CCC without extract; 2, extract without CCC; 3, extract after protein precipitation ($ZnCl_2$) + CCC; 4, untreated extract + CCC; 5, dialyses water + CCC; 6, dialysed extract + CCC; 7, dialysed water + dialysed extract +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

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.

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.

M. M. EL-FOULY and J. JUNG

Botany Laboratory, National Research Centre, Cairo (Dokki, U.A.R.), and Agriculture Experimental Station, Badische Anilin- und Sodafabrik, A.G., Ludwigshafen (W. Germany), 21 November 1968.

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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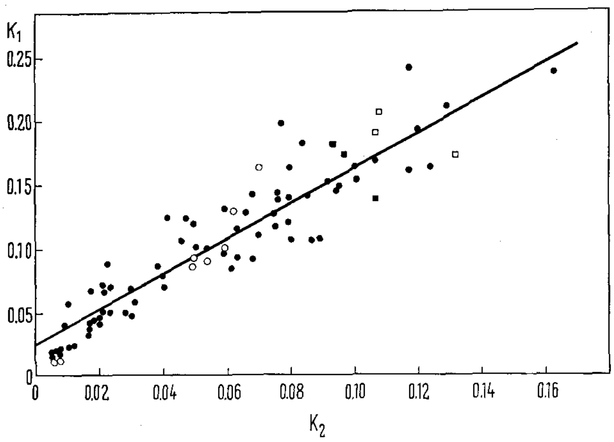

Fig. 1. Illustrates the relationship between the first phase of excretion (K_1) of BSP and the second phase (K_2) in cattle in which hepatocellular damage was induced. ●—●, yellow-wood poisoning; ○—○, lantana poisoning; ■—■, chloroform poisoning; □—□, tanbark poisoning.

ficients for these lines were $r = 0.9135$ and 0.9171 respectively. These correlation coefficients were both highly significant ($P < 0.001$). The similarity between the lines for sheep and cattle should be noted. In fact it could be held that with a larger number of observations for sheep the lines would be identical for these species.

It is apparent from these results that with all the disease conditions examined in both sheep and cattle, the value of K_1 is related to that of K_2, thus suggesting that in each of these disease conditions one or more of the functions of storage, conjugation or excretion of BSP is important in limiting the removal of the dye from the circulation.

It is interesting to point out that K_2 is always much lower than K_1 and in fact that a second phase (K_2) can be associated with values of K_1 which are definitely within the normal range. This means of course, that values of K_2 are a more sensitive test of liver function than those of K_1. From a practical point of view, it could be suggested that in order to reduce the number of blood samples in routine BSP clearances for clinical purposes, perfectly valid results could be obtained by

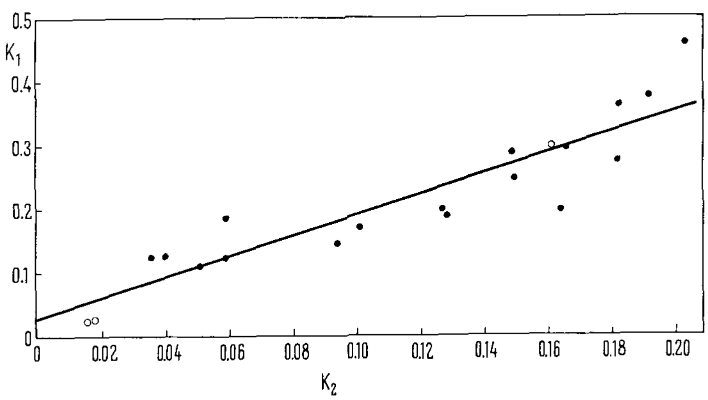

Fig. 2. Illustrates the relationship between the first phase of excretion (K_1) of BSP and the second phase (K_2) in sheep in which hepatocellular damage was induced. ●—●, chronic copper poisoning; ○—○, chloroform poisoning.

the bile. This can occur in the normal animal by increasing the load of BSP presented to the liver (e.g. by increased hepatic blood flow or a higher dose rate of BSP[27–29]) or in abnormal animals, by reducing the efficiency of one or more of the mechanisms involved in the excretion of BSP after it is removed from the circulation. The latter situation appears to have existed in the disease condition 'Yellow-wood poisoning' studied by HUNT and McCOSKER[22]. One or more of the mechanisms of storage, conjugation or excretion has limited the removal of BSP from the circulation initially.

If the assumption that the value of the first phase of the excretion curve (K_1) is determined to a large extent by the mechanisms involved in the second phase of excretion is correct, it should be possible to establish a correlation between K_1 and K_2. In order to test this assumption, values of K_1 were plotted against K_2 for 27 cattle and 6 sheep in which hepato-cellular damage had been induced by a variety of toxic agents. In the case of cattle the 82 results comprised 68 yellow-wood (*Terminalia oblongata*) poisoning, 8 lantana (*Lantana camara*) poisoning, 3 tanbark (*Acacia decurrens*) poisoning and 3 chloroform poisoning. The 20 sheep results represent 17 chronic copper poisoning and 3 chloroform poisoning. The line of best fit for each of these plots was calculated and for cattle was shown to be $K_1 = 1.4\ K_2 + 0.025$ and for sheep was $K_1 = 1.6\ K_2 + 0.030$. The correlation coef-

estimating the fractional clearance of K_2 only. This could be achieved in cattle by collecting specimens at 12, 16 and 20 min after injection of BSP[30].

Zusammenfassung. Ein direkter Zusammenhang zwischen der ersten Phase (K_1) und der zweiten Phase (K_2) der fraktionellen Senkung des Bromsulphthaleinspiegels hat sich an von hepatoxischen Krankheiten befallenen Kühen und Schafen erwiesen. Es ist anzunehmen, dass die K_2-Werte empfindlichere Nachweise der Lebertätigkeit in diesen Tieren aufweisen als die K_1-Werte.

S. E. HUNT and P. J. McCOSKER

Department of Veterinary Clinical Studies, University of Queensland, Veterinary School, Brisbane (Queensland, Australia), 6 January 1969.

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[30] Acknowledgments. We wish to acknowledge the support of the Australian Meat Research Committee for this work and the assistance of Dr. A. SEAWRIGHT, Dr. E. W. MOODIE and N. DOYLE. The interest and encouragement of Professor P. B. ENGLISH is also acknowledged.