pH_e and pH_i at 4 °C of red cells in ACD medium

Days of storage	Hematocrit value	Total water content (%)	DMO in suspension/ DMO in suspernatant	pH_e	pH_i
1	46.4	80.0	0.85	7.50	7.61
2	46.3	79.6	0.85	7.46	7.55
4	46.4	80.0	0.83	7.42	7.47
6	45.6	80.0	0.85	7.35	7.44
8	46.3	79.8	0.85	7.34	7.39
10	45.8	80.0	0.86	7.30	7.40
12	45.8	80.0	0.86	7.28	7.39
14	46.1	80.0	0.88	7.19	7.33
17	46.7	79.9	0.90	7.12	7.28
19	46.5	79.9	0.91	6.99	7.18
22	46.0	79.9	0.92	6.98	7.20
25	45.5	79.9	0.91	6.80	7.06
28	45.0	79.7	0.94	6.84	7.12

buffered saline. However, as shown in the present study, the pH_i was higher than the pH_e in ACD blood, which was further confirmed by freezing and thawing of ACD-stored packed red cells covered with liquid paraffin. The pH at 4°C of the red cells increased from 7.29 to 7.39 by hemolysis. The observation that the pH_i of ACD blood is higher than the pH_e can be explained by Gibbs-Donnan equilibrium based on the impermeability of citrate ion to the cell membrane[5]. The increase of the difference between the pH_i and the pH_e observed during the storage can be explained by the acidification of the suspension and is not due to the aging of the cells. The similar increase was observed when fresh ACD blood was acidified with lactic acid.

The characteristics of the glycolytic reaction in ACD blood are determined by at least 2 factors: pH_i and low temperature. Although data are available about the effect of the pH_e on the glycolysis at 37°C[6], no data are available about the effect of the pH_i. On the other hand, as the pH_i is extremely susceptible to temperature change, the data obtained at different temperature[7,8] need to be reconsidered in relation to the shift of the pH_i during the temperature change. Improvement of blood preservation method may be attained by examination of the pH_i of red cells in different storage medium and the effect of the pH_i on the glycolysis.

Zusammenfassung. Nachweis, dass in ACD-Blut das intrazelluläre pH höher ist als das extrazelluläre pH und das es während der Lagerung bei 4°C langsamer sinkt.

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Effect of Non-Narcotic Analgesics on Anticoagulant-Induced Hypoprothrombinemia in Rats

It has been suggested by several writers[1,2] that salicylates potentiate the action of oral anticoagulants. High doses of acetylsalicylic acid (ASA) have been shown to augment hypoprothrombinemia in humans[3]. Similar effects have heretofor not been demonstrated in other species. An earlier report[4] from our laboratory showed that a single oral dose of 100 mg/kg of ASA decreased the hypoprothrombinemia induced in male rats by *bis*hydroxycoumarin (BHC). The results of further studies on the pharmacologic interaction of analgesics and oral anticoagulants in rats are described in the present communication.

Following the procedures described earlier[4], the effect of oral administration of ASA, 100 mg/kg, daily for various periods up to 35 days on the prothrombin time of blood was investigated in male and female adult Wistar rats treated with BHC by the oral and intraperitoneal routes. The results are summarized in Figure 1. ASA decreased the hypoprothrombinemic effect of BHC in both sexes and there is no indication that chronic administration increased the magnitude of the anti-BHC action of the analgesic. The time course of this effect is illustrated in Figure 2. It becomes significant 18 h after ingestion of ASA; after 24 h the prothrombin time of the BHC-treated groups is still elevated while that of the animals administered both drugs has returned to normal levels.

Treatment	Day 1	Day 2	Day 3
BHC	20 mg/kg i.p.	15 mg/kg i.p.	15 mg/kg i.p.
BHC+	20 mg/kg i.p.	15 mg/kg i.p.	15 mg/kg i.p.+
ASA	—	—	100 mg/kg orally
ASA	—	—	100 mg/kg orally

Tail blood was taken on Day 3 at time intervals, commencing 2 h after the drugs were administered. Each point represents the mean ± standard error of determinations on blood from 6 animals. * $P < 0.05$.

Similar experiments were conducted with a single dose of the following compounds: sodium salicylate (88.9 mg/kg), phenacetin (50 mg/kg), phenylbutazone (10 mg/kg), acetaminophen (50 mg/kg), salicylamide (100 mg/kg), acetanilide (50 mg/kg), and antipyrine (50 mg/kg). Sodium salicylate reduced the prothrombin time of BHC-treated animals from 19.7 ± 1.8 sec to 13.1 ± 0.3 sec ($P < 0.005$). Phenylbutazone appeared to have a similar but less pronounced effect ($P < 0.10$). None of the other drugs examined altered the prothrombin time.

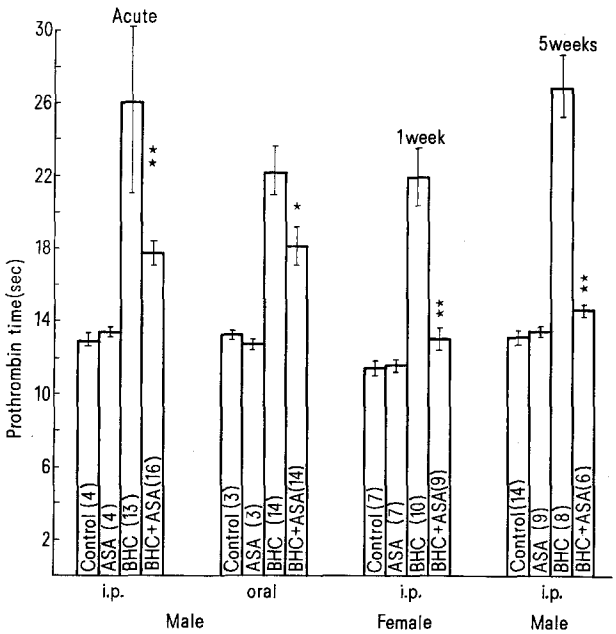

Fig. 1. Effect of acetylsalicylic acid (ASA) and bishydroxycoumarin (BHC), separately and in combination, on the prothrombin time of blood taken from rats 18 h after final drug administration. The numbers in parentheses indicate the number of animals in each group. Treatments were as follows: ASA, 100 mg/kg, orally, as a 0.5% suspension in 0.25% aqueous gum tragacanth, as a single dose (acute) or daily as indicated. BHC, a loading dose of 20 mg/kg followed by a maintenance dose of 15 mg/kg per day for 2 days in the acute and 1 week experiment and for 6 days in the 5 week experiment, by the i.p. and oral routes indicated. Prepared fresh daily in 0.015 N NaOH, 10 mg/ml. $*P < 0.05$; $**P < 0.01$.

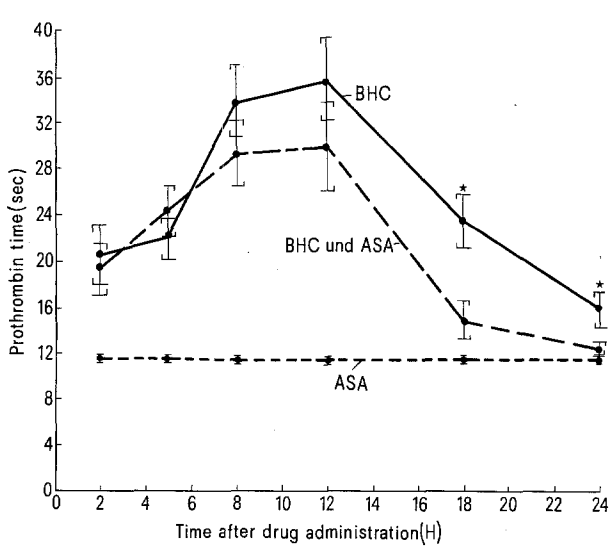

Fig. 2. Prothrombin time of tail blood taken at periodic intervals after treatment of rats with bishydroxycoumarin (BHC, —) and acetylsalicylic acid (ASA, ---) separately, and in combination (BHC+ASA, ——). The treatments were as follows:

An experiment was conducted to determine if ASA would alter the hypoprothrombinemic effect induced by warfarin and another synthetic anticoagulant, phenindione, which is similar in action to BHC but chemically unrelated. Investigation showed that an oral dose of 1.0 mg/kg of warfarin increased the prothrombin time from the normal value of 12.3 ± 0.14 sec to 29.2 ± 1.59 sec, measured 18 h after ingestion. This was reduced to 22.7 ± 1.22 sec ($P < 0.01$) when 100 mg/kg of ASA was administered with the anticoagulant. The therapeutic dose of phenindione was difficult to establish because of the variability in the response of the individual animals. The intraperitoneal administration of 40 mg/kg of phenindione in 0.25% gum tragacanth daily for 3 days increases the prothrombin time from the control value of 13.7 ± 0.08 sec to 18.1 ± 1.25 sec, representing approximately 20% of the prothrombin activity[4], still present. This value was unchanged by the oral administration of ASA.

The reason for the decreased prothrombin time in the rat observed in the above experiments is not readily apparent. While salicylate prolongs the prothrombin time of most normal human subjects, it has a relatively slight effect on the prothrombin time of patients on anticoagulant therapy[5,6]. Phenylbutazone potentiates the action of BHC in man but reduces it in the dog[7]. This action has been ascribed to the fact that phenylbutazone competes with BHC for plasma protein binding sites, thereby increasing the rate of delivery of the anticoagulant to the liver and thus the effective hepatic concentration. It has been suggested that in man this effect on transport appears to outweigh the phenylbutazone-mediated stimulation of BHC metabolism in the liver whereas in the dog the reverse seems to be the case. However, the possibility that salicylate and phenylbutazone may compete with BHC for the receptor sites of the latter in the liver cannot be excluded. Thus the effect of these drugs on the pharmacological action of BHC in man and rat may be related to species differences in affinity for plasma proteins and receptor sites and in their pharmacokinetics. Presently, these possibilities are being investigated in our laboratory[8].

Résumé. Chez le rat, un traitement à l'acide acétylsalicilique, au salicilate sodique et au phénylbutazone fait remonter le taux de protrombine abaissé par la bishydrocoumarine.

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