($p < 0.05$) but a 75% ($p < 0.01$) increase in glycogen utilization was observed with 55 mM aspirin. In contrast sodium salicylate appeared to have no significant effect on glucose uptake even at 55 mM ($p > 0.05$) but increased glycogen utilization by 44% at 5 mM ($p < 0.01$) and by 69% at 55 mM ($p < 0.01$) (Figure 2b).

The results indicate that aspirin and sodium salicylate have different effects on platelet glucose uptake but they have similar effects on platelet glycogen utilization. However since therapeutic levels of aspirin or salicylate do not normally exceed 0.5 mM it is unlikely that either of these drugs would effect platelet glycogenolysis in vivo. On the other hand the observation that aspirin will reduce glucose utilization at a concentration of 0.05 mM suggests that this effect may occur in vivo following the administration of aspirin in therapeutic doses. This decrease may arise from an inhibitory effect of aspirin on an ATP requiring platelet process such as the energy dependent ADP release which occurs when platelets in washed suspension are exposed to surface stimuli such as the incubating container[13]. Alternatively aspirin may produce this effect by inhibiting glucose uptake either directly or through an effect on glycolysis. This latter effect is unlikely since the rate of glycogen depletion was unchanged and previous studies failed to demonstrate an effect of aspirin on hexokinase activity[7].

The results of these and other studies suggest that aspirin has two types of effects: 1. those arizing from the acetyl grouping which include inhibition of the platelet release reaction[8] and glucose uptake and 2. those arizing from the salicylate moiety which include stimulation of glycogenolysis and uncoupling of oxidative phosphorylation[13,15].

Zusammenfassung. Es wird festgestellt, dass therapeutische Konzentrationen von Aspirin, die die Reaktivität der Blutplättchen hemmen, mit einer signifikanten Herabsetzung der Glukoseverwertung einhergehen. Natriumsalicylat, das keine Acetylgruppe enthält und die Aggregation der Thrombocyten nicht beeinflusst, besitzt dagegen keinen solchen Effekt.

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The Role of Hypercapnia in Acetazolamide Teratogenesis

The carbonic anhydrase inhibitor acetazolamide, when given in large amounts to rats, mice[1] and hamsters[2] during pregnancy, produces malformations almost exclusively of the postaxial portion of the forelimb. The mechanism of teratogenesis is of interest because the lesion is quite specific, and because it apparently involves carbonic anhydrase inhibition[3,4] at an intrauterine site[5,6]. Since a teratogenic dose of acetazolamide raises the tissue pCO_2[7], we tried to imitate the teratogenic effect of acetazolamide with CO_2 enriched air. The possibility of interaction between CO_2 exposure and acetazolamide was also examined. We were aware that HARING[8], using an atmosphere enriched in CO_2 to produce cardiac malformations in rat embryos, reported no limb malformations. But, because the acetazolamide sensitive period and dose response relationship have been thoroughly worked out for the hamster[2], we felt CO_2 exposure during the specific acetazolamide sensitive period was warranted.

Materials and methods. Random-bred, virgin golden hamsters *Cricetus auratus* were mated under direct observation. They were allowed food and water ad libitum. 204 h later, the animals were exposed for 8 h to an increased tension of CO_2 by placing cages in a gas-tight chamber ventilated with CO_2 enriched air. The gas mixture in the chamber was kept at atmospheric pressure, and the concentration of CO_2 in the exhaust was continuously monitored[9].

The plan of treatment is shown in the Table. Some animals were exposed to either 4% or 10% CO_2. Other animals were given the sodium salt of acetazolamide, 600 mg/kg i.p., and then immediately placed in the CO_2 enriched atmosphere. Another group of animals was given a single peritoneal injection of sodium acetazolamide, 600 mg/kg, at 204 h after mating with no additional treatment. All animals were killed approximately 280 h after mating. The fetuses were examined for external malformations, particularly the limbs. The statistical significance of differences between experimental groups was determined by using tables of binomial confidence limits[10]. As used here significant differences refer to confidence limits of 95% or greater.

Results. There were no externally deformed fetuses from the group exposed to 4% CO_2. In the group exposed to 10% CO_2, 2 fetuses with external malformations were found. One had a postaxial deformity of the right forelimb, consisting of a fifth digit perpendicular to the axes of the remaining digits. The other had a preaxial deformity of the right forelimb, consisting of an abnormal angulation of the tip of the second digit.

All external embryonic malformations from the group treated with acetazolamide and 4% CO_2 were confined

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Incidence of deformities in fetuses from hamsters exposed to CO_2 enriched air from 204–212 h after mating, alone, and combined with single i.p. dose of acetazolamide at 204 h after mating

Treatment			Resorptions	Malformed fetuses			Postaxial forelimb defects	
CO_2 in air (%)	Acetazolamide (mg/kg)	No. of dams	Total implantations (%)	Number Total	%	Bilateral forelimb defects (%)	No. of limbs	With ectrodactyly (%)
4	0	6	9	0/53	0	0	0	0
10	0	15	6	2/171	1.2	0	1	0
4	600	6	9	23/64	36	61	37	54
10	600	12	7	65/118	55	72	108	73
0	600	44	7	271/629	42	53	403	64

to the postaxial parts of the forelimb. The incidence of deformed fetuses, the frequency of bilateral forelimb involvement, and the incidence of ectrodactyly (an indication of the severity of the lesion[2]) were not significantly different from that caused by acetazolamide alone. In the group treated with acetazolamide and 10% CO_2, the fetal malformations were limited to the postaxial parts of the forelimbs. The incidence of deformed fetuses and the frequency of bilateral involvement, but not the incidence of ectrodactyly, were significantly greater than that caused by acetazolamide alone.

The incidence of resorptions in any CO_2 exposed group was not significantly different from the group receiving acetazolamide alone.

Discussion. The CO_2 exposures of this study produced tissue pCO_2's comparable to that produced by a teratogenic dose of acetazolamide. The mixed venous pCO_2 of hamsters breathing 4% CO_2 was calculated to be 58 Torr, and with 10% CO_2, 91 Torr[11–15]. The pCO_2 of the acetazolamide treated hamsters can be approximated by values for rats, in which 50 mg/kg of acetazolamide resulted in a gas pocket pCO_2 of 57 Torr[7]. A larger dose, such as the 600 mg/kg of this study, would not increase the pCO_2 further[16,17], although it would prolong its elevation. The duration of CO_2 exposure was probably adequate, since postaxial defects can be observed grossly 24 h after acetazolamide administration[2].

The results show that the teratogenicity of acetazolamide is not due solely to its effect in raising the CO_2 tension. The 2 limb deformities that were found in the group exposed to 10% CO_2 probably resulted from a non-specific deleterious effect acting during early limb morphogenesis. The preaxial limb malformation is not the kind produced by acetazolamide and the postaxial deformity, although the hallmark of the acetazolamide effect, has occasionally been seen with other teratogens such as 5 fluorouracil[18] and riboflavin-deficient galactoflavin-containing diets[19].

On the basis of this study, we cannot explain how 10% CO_2 interacted with acetazolamide to increase the incidence of deformities over that caused by acetazolamide alone. Perhaps exposure to 10% CO_2 acidified the urine, leading to slower renal elimination of acetazolamide[20]. Consistent with this explanation is the finding[2] that increasing doses of acetazolamide increase the incidence of forelimb malformations and the frequency of bilateral involvement, but not the incidence of ectrodactyly or the rate of fetal resorption[21].

Zusammenfassung. Für die teratogene Wirkung von Acetazolamid beim Hamster ist nicht nur der die CO_2-Konzentration erhöhende Effekt verantwortlich. Das Aussetzen der mit Acetazolamid behandelten Muttertiere in eine mit 10% CO_2 angereicherte Atmosphäre erhöhte zwar die Inzidenz, nicht aber den Schweregrad der Missbildungen der Vorderextremität.

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