Table II. The volume of hind paws in ml before (A) and 120 min after (B) i.p. administration of edema producing agents

Treatment (No. of experiments)	Vol. of the hind paws in ml	Inhibition in % of control value	P
Dextran, control (40)	A 0.408 ± 0.016 B 0.609 ± 0.046	–	–
Dextran, sham operation (20)	A 0.401 ± 0.016 B 0.574 ± 0.015	14	$\cong 0.05$
Dextran, nephrectomy (20)	A 0.402 ± 0.018 B 0.461 ± 0.051	71	< 0.001
Dextran, nephrectomy, insulin (20)	A 0.381 ± 0.018 B 0.506 ± 0.044	38	< 0.001[a]
Egg white, control (20)	A 0.394 ± 0.010 B 0.544 ± 0.060	–	–
Egg white nephrectomy (10)	A 0.382 ± 0.014 B 0.416 ± 0.023	77	< 0.001

[a] Compared with the nephrectomized group.

evoking effect of 5-HT remains nearly unaltered after nephrectomy, it may be supposed that the susceptibility of peripheral receptors to the mediator substances does not change. We suppose that the inhibition develops in an earlier phase of the edema-producing processes.

The elevation of the BUN probably does not play a role in the prevention of the AE, as shown in our experiments in which urea administered i.p. in a dose of 200 mg/kg body weight was not able to prevent the formation of AE.

Zusammenfassung. Nephrektomie hemmt bei Ratten die Ausbildung des lokalen sowie des generalisierten Anaphylaktoid-Ödems erheblich. Die Wirkung der lokal injizierten Substanz 48/80 wird ebenfalls gehemmt, während der Effekt des in die Pfote verabreichten Serotonins unverändert bleibt. Insulin antagonisiert den Hemmungseffekt der Nephrektomie.

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Inhibition of the Morphogenesis of the Otoliths in the Chick Embryo in the Presence of Carbonic Anhydrase Inhibitors

The otoliths in the chick are composed of calcium carbonate in the form of calcite[1] and of an organic matrix which is formed in large part of acid mucopolysaccharides[2,3] and of protein which does not have the classical collagen structure[4].

The otoliths begin to differentiate in the chick at about the fifth day of incubation. The mode in which their morphogenesis is actuated is still obscure. Autoradiographic observations[5] have revealed a precocius localization and an active turnover of ^{45}Ca at the level of the endolymphatic sac and of the pars inferior of the labyrinth which gives rise to the sacculus and the lagena.

This paper reports the results of experiments[6–9] conducted with sulphonamides which are specific inhibitors of carbonic anhydrase.

Material and methods. The following sulphonamides were used: acetazolamide (2-acetylamino-1, 3, 4-thiadiazole-5-sulphonamide), dichlorphenamide (1-3-disulphamyl-4-5 dichlorobenzene), ethoxyzolamide (6-ethoxybenzothiazole-2-sulphonamide) and neptazane (5-acetylimino-4-methyl-Δ^2-1, 3, 4-thiadiazoline-2-sulphonamide).

The sodium salt of acetazolamide and dichlorphenamide (lyophilized) were dissolved in double distilled water and physiological solution respectively, ethoxyzolamide and neptazane were dissolved in dimethylsulphoxide (Fluka). The controls were injected with double distilled water, physiological solution and dimethylsulphoxide. The experimental and control solutions were injected into the egg albumen on the fourth day of incubation (Lillie stage 23[10]), a stage at which the morphogenesis of the otoliths has not initiated, but which immediately preceeds the initiation of active turnover of ^{45}Ca at level of the membranous labyrinth.

According to CONTI and MILIO[11], 0.01 ml of the solutions were injected. The embryos were fixed at different stages during development in neutral formalin, the heads were embedded in paraffin and sections were treated with the PAS reaction or were stained with alcian-PAS[12].

Results. The results of the various experiments are reported in the Table. The doses indicated in the Table are those which did not have a high toxic action; the mortality was only a little higher than that reported for the controls by CONTI and MILIO[11].

An examination of the Table shows that the substances used cause an alteration in the otoliths only, in which morphogenesis is inhibited in most cases.

The macular ephithelium is normal as are the other structures of the membranous labyrinth (cristae, maculae,

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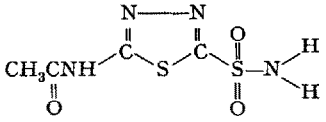
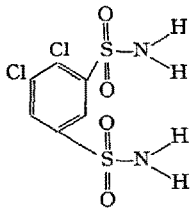
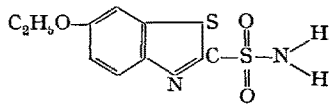
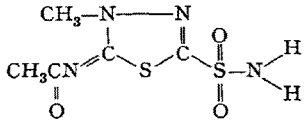
	Days of development at which the embryos were sacrificed	Embryos which survived treatment	Presence of the otoliths in both labyrinths	Presence of the otoliths in one labyrinth	Absence of the otoliths in both labyrinths
Control embryos (treated with double distilled water)	8	8	8	–	–
Control embryos (treated with physiological solution)	8	9	9	–	–
–	13	4	4	–	–
–	18	4	4	–	–
Control embryos (treated with dimethylsulfoxide)	8	8	8	–	–
Embryos treated with acetazolamide 					
3 mg/egg	8	16	10	6	–
4 mg/egg	8	10	2	4	4
6 mg/egg	8	12	1	1	10
Embryos treated with dichlorphenamide 					
2 mg/egg	8	31	3	4	24
2 mg/egg	13	5	–	–	5
2 mg/egg	18	2	–	–	2
4 mg/egg	8	10	–	–	10
Embryos treated with ethoxyzolamide 					
2 mg/egg	8	10	4	1	5
2 mg/egg	13	5	2	–	3
4 mg/egg	8	3	1	1	1
Embryos treated with neptazane 					
2 mg/egg	8	14	5	1	8
4 mg/egg	8	2	–	–	2
6 mg/egg	8	1	–	–	1
8 mg/egg	8	3	–	2	1

organ of Corti). In some cases the absence of the otoliths is observed only on one side while in other cases its morphogenesis is limited to the presence of a few calcified granules which tend to unite sometimes forming a single large otolith which in this case is usually localized in the sacculus of one side. The histochemical reaction for carbonic anhydrase which in the embryo at the V day of development is restricted to the epithelium of the endolymphatic sac[13] is negative after the injection of the inhibitors used[14].

Discussion and conclusion. The results reported above would indicate that carbonic anhydrase plays a role in the

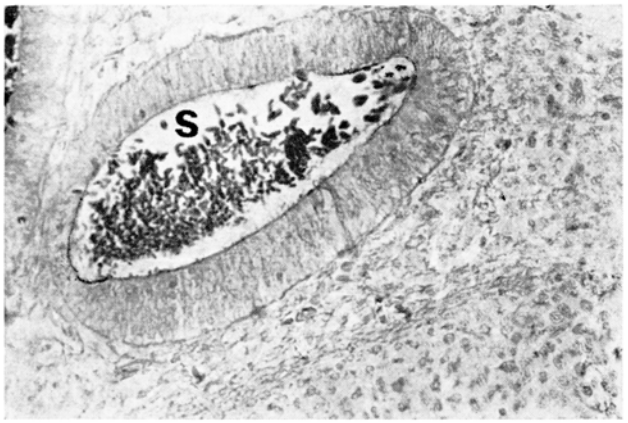


Fig. 1. 8-day chick embryo: bud of sacculus (S). Presence of otoliths. × 128.

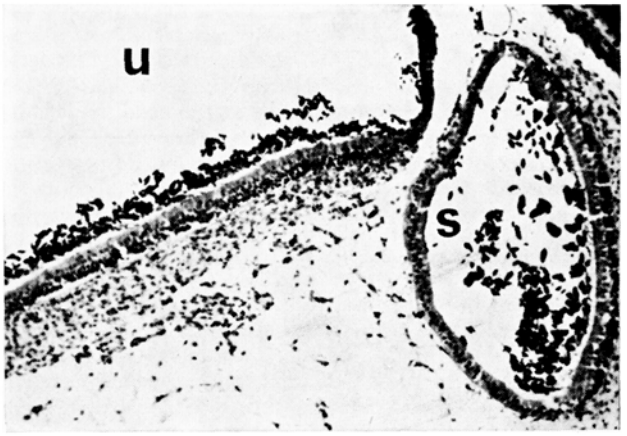


Fig. 3. 13-day chick embryo. Section of membranous labyrinth. Presence of otolithic membrane at the level of utricular (U) and saccular (S) maculae. × 128.

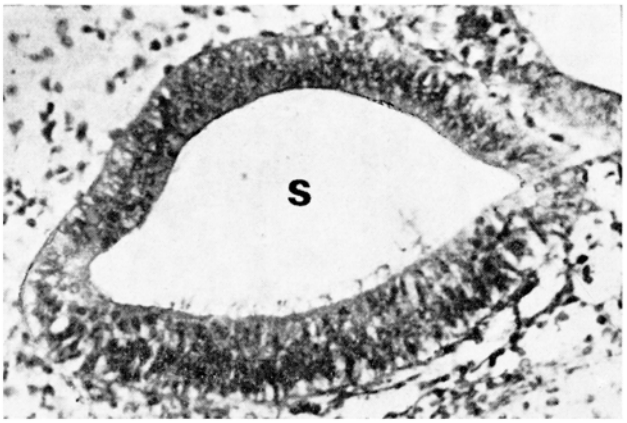


Fig. 2. 8-day embryo (treated with 6 mg of acetazolamide sodium): bud of sacculus (S). Absence of otoliths. × 128.

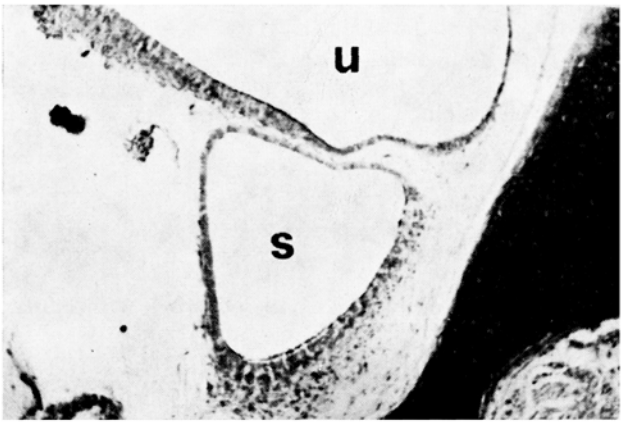


Fig. 4. 13-day chick embryo (treated with 2 mg of dichlorphenamide). Section of membranous labyrinth. Absence of otoliths at the level of utricular (U) and saccular (S) maculae. × 128.

morphogenesis of the otoliths in chick embryos. In fact, using various inhibitors of this enzyme one observes a selective action, during the morphogenesis of the structures of the otoliths. The inhibitors used, which vary in the radical, all have in common the free sulphonamide group-SO_2NH_2 which many authors believe to be the group active in carbonic anhydrase inhibition[15-20].

In the chick the action of carbonic anhydrase has been reported in the formation of the shell[21]; in echinoderms[22] and molluscs[22-24] carbonic anhydrase has an important function in the process of calcification.

The action of carbonic anhydrase in the morphogenesis of the otoliths is effective at the moment at which the turnover of ^{45}Ca is high at the level of the membranous labyrinth[5]. In this period, there would be a greater need for an enzyme which is known to accelerate the reaction $H_2O + CO_2 \rightleftarrows H_2CO_3$, a reaction which takes place at a low rate even without the intervention of carbonic anhydrase[23].

Riassunto. Vengono studiati gli effetti sullo sviluppo del labirinto membranoso dell'embrione di pollo di alcuni inibitori della anidrasi carbonica (acetazolamide, dichlorofenamide, etossizolamide, neptazano). L'azione di tali sostanze si manifesta con una elettiva inibizione della morfogenesi degli otoconi.

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