gauze and containing 200 ml of water at 30–40 °C contaminated with approximately 200 cercaria. Exposure was for 3 h. After 6 weeks, 24 h stools were examined for positive infection through an egg count. Each mouse was kept separate. Mice producing stools containing living, hatchable ova were isolated. The hatchability was examined, through the addition of an excess of warm water to the egg concentrate obtained through centrifugation. 25 infected mice were divided into 3 groups: (A) 5 were kept as controls. (B) 5 were given lucanthone (*M* weight 370) in a dose of 20 mg/kg twice daily at a 6 h interval from a water solution (100 mg%) for 15 days. (C) 15 were subdivided into 3 subgroups, each containing 5 mice. Each mouse was administered 20 mg/kg lucanthone, followed by 15 mg/kg of sulphamethazine for the first group, 13.6 mg/kg sulphadiazine for the second and 13.5 mg/kg sulphathiazole for the third. The combined doses were given twice daily at a 6 h interval for 15 days. Follow-up of the egg excretion began right after administration periods for the 3 groups in 24 h stools.

Results and discussion. The A (control) group continued egg excretion for the whole period of examination (4 weeks) parallel to the other 2 groups.

The B group (given lucanthone alone) showed continuous depression of the egg count, and 3–4 weeks after discontinuation of drug administration no more ova could be detected.

The ova count for the members of group C (those administered the sulphonamide together with lucanthone) did not show a depression in the egg count. Living ova were present during the 4-week examination period.

When the animals were sacrificed by decapitation 120 days after administration of either lucanthone alone or lucanthone and sulphonamides, the following observations were recorded. In group A (control), excretion of eggs continued during the period before dissection. Living worms were found in the mesentric venules. In group B, excretion of eggs continued during the entire period before dissection (120 days after the end of administration of drugs). Living worms were found in the liver. This meant an hepatic shift had occurred. In group C, containing animals given lucanthone alone, excretion of eggs ceased 3–4 weeks after stopping drug administration, and after 120 days, when the animals were dissected, in one mouse no worms were found while the other 4 contained dead worms in the liver.

This suggests that lucanthone, with or without sulphonamide, causes hepatic shift of worms from the mesenterics, and the addition of sulphonamide antagonizes the therapeutic action of lucanthone in the liver. These results support our earlier hypothesis that peroxidase is involved in the chemotherapeutic effect of lucanthone[3].

Zusammenfassung. Heterocyclische Sulfonamide, in äquimolekularer Menge an mit *Schistosoma mansoni* infizierte Mäuse verabreicht, heben die Hemmung von Lucanthone auf die Entwicklung des Parasiten auf.

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Further Observations on Age Differences in the Effects of Formalin on the Canine Brain in vitro

The effects of formalin fixation on the brain of the Macaque, man and small mammals have been studied by several investigators[1–5] and recently reported in a preliminary study on the dog[6]. Fox[7] demonstrated that there is a linear decrease in the percentage weight gain of whole brain after 24 h in 20% formalin from 47.5% at birth to 15% in the young adult dog, and a decrease in total dry matter content in the cerebrum and medulla[6,7]. The present investigation was conducted to ascertain possible age differences in the effect of formalin fixation on the major cortical regions of the canine brain during postnatal growth.

9 dogs at selected ages were deeply anesthetized with intravenous pentobarbital, and following exsanguination by section of carotids and venae cavae the anemic brains were removed and dissected into appropriate regions and treated as follows:

(1) *Age differences in effect of formalin.* The cerebral cortices of 8 dogs aged 1 day, 1, 2, 3, 4, 12, 16 weeks and adult were dissected and approximately 1 g of tissue was taken from the frontal lobe of each hemisphere in coronal section. The tissue was first weighed on a Mettler shadowgraph balance before immersion in 40% formalin (1 g tissue/30 ml). The formalin was changed daily to insure a constant concentration, and tissues weighed after wiping lightly on filter paper on days 1, 4 and 7.

(2) *Edema properties of the developing brain.* 9 subjects aged 1 day, 1, 2, 3, 4, 7, 12 and 16 weeks and adult were studied (8 of these were used from part 1 after frontal lobe dissection). The parieto-temporal region of the cerebrum was removed, and as in the case of the formalin fixation studies, double samples of similar weight from opposite cortical lobes were studied in each specimen. The parieto-temporal region on each side was dissected into 3 segments, and, after weighing, these segments were placed respectively in normal saline (0.9% NaCl) half normal saline and twice normal saline (1 g tissue/30 ml). Weights were taken after 24 h immersion to determine the increase in weight. Results were averaged from double samples of each age group studied, and all specimens were maintained at 28 °C (± 1 °C) in airtight vials throughout these experiments.

The effects of formalin fixation (Figure 1) involve a rapid increase in the weight of the brain during the first day of immersion followed by a rapid decline in weight, so that by the fourth day considerable shrinkage has

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taken place. From 4–7 days of age the decrease in weight is more gradual. The age differences in the effect of formalin on the cerebral tissue is most obvious during the first day of fixation, increase in weight being greater in younger brains. The dramatic effect of formalin, initially causing swelling and later shrinkage, is less marked in older specimens (Figure 1).

The age differences in the edema properties of the cerebral cortex is clearly demonstrated (Figure 2). Only in new-born animals was there a significant weight increase, and these findings suggest that the brain of the new-born is extremely hydrophilic, while subjects from 1 week of age onward did not show such a marked change following immersion in saline of different osmotic strengths. In all age groups the degree of edema bore a constant relationship to the concentration of saline used (Figure 2).

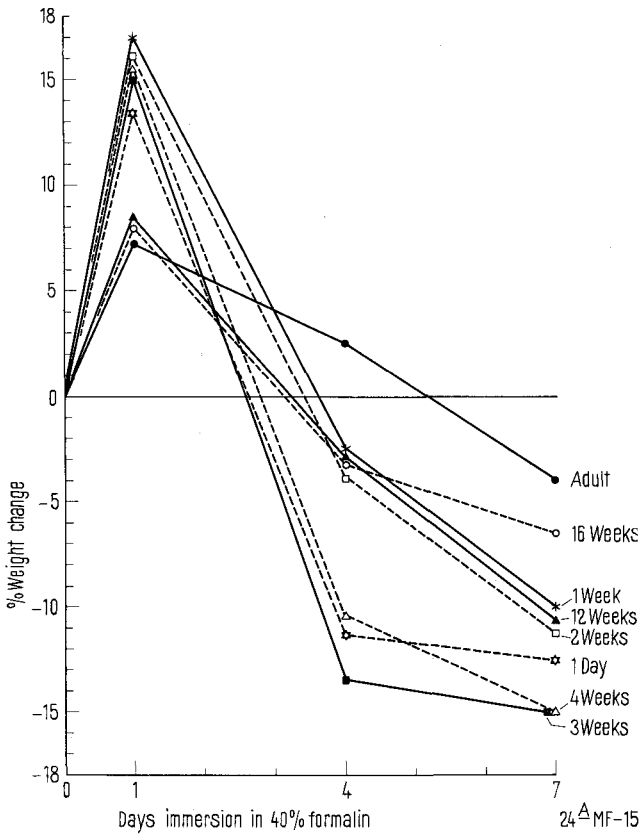

Fig. 1. Age differences in effect of formalin on dog cerebrum.

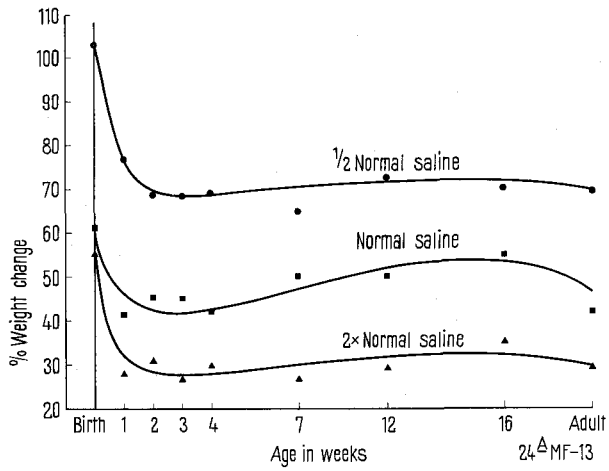

Fig. 2. Weight differences with age of cerebral edema in vitro.

These findings suggest that, in formalin, the more immature brains undergo greater physical changes (swelling followed by shrinkage); in saline, however, the edematous potential of the tissue is confined approximately to the immediate postnatal period. Formalin fixation clearly has a marked effect on the dry matter content of the brain irrespective of the age of the subjects, causing a reduction in the percentage dry matter which may be due to swelling and leaching of formalin soluble elements[6]. CUMINGS[8] reported that phospholipids are lost in formalin, and it is possible that several of the lipid constituents of the brain are leached during fixation. KIYOTA[9] suggested that the hydrophilic properties of the brain are greater in younger animals where the albumen content is higher; soluble proteins decrease with age and these proteins have a very high hydration, so that young brains may retain more water. Each region of the CNS showed individual variation in capacity to swell or shrink following formalin fixation[10]. With higher concentrations of formalin there is less weight increase, and weight change is a function of time of fixation and fixative strength[11]; no marked differences in cell density or volume of similar cortical areas fixed in different concentrations of formalin was found. MANN[12], in discussing the effects of formalin on serum albumen, reported that after long exposure albumen will precipitate on heating instead of coagulating, but if water is then added to this precipitate it will dissolve (i.e. reversible effect with hydration). The effects of formalin on proteins must be considered; proteins swell and then shrink, also there is considerable exchange of water from cell and tissue spaces. Thus, the in vitro edema properties of the developing brain and swelling and shrinkage effects of formalin decrease with age, reflecting changes in total protein and hydrophilic albumen.

Zusammenfassung. Die Wirkung des Formols (40%) auf spezifische Areale der Gehirnrinde von Hunden verschiedenen Alters wurde in den ersten 7 Tagen der Fixation studiert. Die Gewichtszunahme nach Eintauchen in Formol und in diverse physiologische Lösungen war altersabhängig. Extrem hydrophile Natur ist für unreife Nervengewebe in vitro typisch.

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