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Influence of Bile Salts on the Nervous System Following Intraspinal Usage.*

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Few observations have been reported concerning the influence of bile and bile salts on nerve tissue.[1] Nerve conduction is not affected but the neuromuscular junction and reflex centers of the cord are influenced by their toxic action.[2] In human subjects it has been claimed that jaundice exercises an analgesic effect on pain.[3] The pruritus of icteric individuals has been linked with a disturbance in the sympathetic nervous system.[4] In both instances, bile salts have been thought to be responsible.

The intraspinal introduction of bile salts was investigated for the purpose of determining whether they exercise an analgesic effect which might be employed for the relief of intractable pain. Clinical experience with the intraspinal use of alcohol for this purpose has been reported by one of us.[5]

Spinal puncture was performed on young lean cats in the lumbar region. Traumatization of the cord at the site of injection frequently occurred judging from the gross pathological findings. In 5 of 105 cats a spontaneously free gush of cerebro-spinal fluid was obtained immediately upon the introduction of the needle. In 28, free fluid could be aspirated. Cisternal puncture was performed in 5 animals.

Motor changes and sensory responses to pin prick were noted at frequent intervals over varying periods of time. Animals were sacrificed under ether anesthesia.

The sodium salt of desoxycholic acid (Riedel-de Haen) was employed because of its purity and great toxicity for pneumococci and erythrocytes.[6]

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1 Biedl, A., and Kraus, R., *Cent. f. Inn. Med.*, 1898, **19**, 1185; Loewit, *Prag. Z. f. Heilk*, 1881, **2**; Cit. by Meltzer, S. J., and Salant, W., *J. Exp. Med.*, 1906, **8**, 127; Pritzker, B., *Dtsch. Z. f. Chir.*, 1934, **243**, 85.

2 Ries, F. A., and Still, E. U., *Am. J. Physiol.*, 1930, **91**, 609.

3 Hench, P. S., *Ann. Int. Med.*, 1934, **7**, 1278.

4 Lichtman, S. S., *J. A. M. A.*, 1931, **97**, 1463.

5 Stern, E. L., *Am. J. Surg.*, 1934, **25**, 217.

6 Lichtman, S. S., *J. Biol. Chem.*, 1934, **107**, 717.

In a control group of cats, 95% ethyl alcohol was introduced into the spinal canal. In a second series, phosphate buffered desoxycholate solutions were used, and in a third, alcoholic solutions of desoxycholate.

95% Ethyl Alcohol. 14 cats were studied in this series. 0.5 cc. could be introduced into the lumbar region with no ill effects. In larger amounts both sensory and motor changes were produced.[7] Via cisternal puncture the injection of 0.1 cc. resulted in slight ataxia lasting 15 minutes, but no gross changes occurred in 13 days. The injection of 0.5 cc. by this route caused prompt respiratory paralysis and death.

Phosphate Buffered Desoxycholate Solutions. Eleven cats were studied in this series. Concentrations between 0.010 and 5.0% in tenth molar phosphate buffer solution pH 7.0, were tested in doses from 0.1 to 3.0 cc. One cc. of 5.0% caused salivation, rapid breathing, incontinence, and death in 30 minutes from respiratory paralysis; one cc. of 0.5% solution caused more marked motor than sensory changes and death in 7 days. 0.5 cc. of 0.5% and of 1.0% solution caused no motor or sensory changes over a period of 14 days.

Desoxycholate in 95% Ethyl Alcohol. Twenty-six cats were studied in this series. Concentrations between 0.010 and 5.0% were injected. 0.1 cc. of 0.010% caused no changes for as long as 15 days. Minimal gross changes were observed on the surface of the cord. Injection of 0.5 cc. of 0.1% caused loss of sensation and motor paralysis of one hind limb and bladder paralysis in one cat. After 10 days a marked hemorrhagic reaction in the lumbar and caudal regions was found. 0.5 cc. of 0.2% desoxycholate caused loss of sensation of all limbs and motor paralysis of the hind limbs, also bladder paralysis. Seven days after injection a hemorrhagic reaction over the entire cord was found.

In cats with an unusually free flow of fluid immediately upon introduction of the needle into the lumbar region, the injection of 0.3 cc. of 2.0% desoxycholate and 5.0% desoxycholic acid did not cause the slightest motor or sensory change. After 30 days no gross change could be detected in the cord. Introduced via cisternal puncture, as little as 0.1 cc. of 0.010% desoxycholate in alcohol caused weakness and ataxia of the hind limbs. After 24 hours a plastic arachnoiditis was found.

Influence of Spinal Fluid and Spinal Cord Tissue on the Hemolytic Action of Bile Salts. Employing the hemolytic action of bile

[7] Stern, E. L., in press.

salts as a possible criterion of toxicity, the influence of the protein content of spinal fluid and of cord tissue upon the action of bile salts was tested. Pooled human spinal fluid, spinal cord of a cat, and a standardized sheep erythrocyte suspension and hemolytic system[7] were used. The presence of spinal fluid in the system necessitated a 40% increase (from 0.010 to 0.014%) of desoxycholate to produce complete hemolysis in a specified time. The further addition of equal segments of spinal cord into the system necessitated a 100% increase, *i. e.,* from 0.010% to 0.020% desoxycholate.

Pathological Findings. Material selected from each of the experimental groups was studied with various staining methods. Where definite fat changes were demonstrable, unequivocal evidence of trauma due to puncture was usually present. In one instance, thrombosis of the anterior spinal artery occurred with extensive myelomalacia in the course of its supply. With alcoholic solutions of desoxycholate in higher concentration, hemorrhagic injection of the meninges up to the cervical region was noted. Localized myelomalacia, softening, and swelling at the level of injection was interpreted as traumatic.

The intraspinal injection of 0.5 cc. of ethyl alcohol alone, in the cat, causes very slight functional and pathological changes in the nervous system. In larger doses it produces sensory and motor changes. Amounts less than 0.5 cc. of buffered desoxycholate in concentrations as high as 1.0% are ineffective in injuring nerve tissue. Combined with as little as 0.1 cc. of alcohol, desoxycholate solutions of 0.025% or more, cause definite sensory, motor, gross and microscopic changes if the lumbar cord is injured at the time of injection. Five percent desoxycholic acid in alcoholic solution produced no injury when there was an immediate free flow of spinal fluid without trauma. Apparently spinal fluid protein and intact nerve tissue tend to diminish the hemolytic activity of the bile salt. The combination of alcohol and bile salt in minute doses is highly toxic to injured nerve tissue. The alcohol apparently permits greater diffusion of the bile salt through injured nerve tissue even in the presence of spinal fluid protein.

The results offer little encouragement for the intraspinal use of bile salt combined with alcohol, or of bile salt alone, for the possible relief of intractable pain. The medullary centers are sensitive to the action of bile salts when introduced by cisternal puncture.

Summary. Sodium desoxycholate can be introduced in minute doses in aqueous solution intraspinally in the lumbar region in cats without untoward effects or injury to the cord. Larger doses pro-

duce motor and sensory disturbances, and even death from respiratory paralysis. Traumatized spinal cord tissue is highly susceptible to the toxic action of even minute doses of bile salt in alcoholic solution although apparently resistant to the same doses in aqueous solution. Spinal fluid protein and cord tissue reduce the hemolytic action of bile salt.

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Digestibility of Gastric Mucin in vivo.

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We have previously reported studies indicating that gastric mucin (hog) is relatively resistant to enzymatic hydrolysis *in vitro*.[1] However, indirect evidence suggests more complete digestion in the gastrointestinal tract.[1] To further investigate the digestion of mucin we have fed purified gastric mucin as the source of nitrogen, to a series of albino rats, and from nitrogen analyses of the urine and feces determined its degree of digestibility *in vivo*.

Our experimental procedures differed little from those commonly employed in the determination of utilization, digestibility and biological value of proteins. Young growing albino rats weighing 40-60 gm. were placed in cages so designed that urine and feces could be collected separately. Nitrogen intake was calculated from the weight of food consumed. The small amount spilled was corrected for by determining its nitrogen content and subtracting from the calculated food nitrogen. All nitrogen analyses were by the Kjeldahl method.

The purified mucin[2] used in the preparation of the mucin diet gave the following analyses: Nitrogen 7.50%. Reduction after acid hydrolysis (Shaffer-Hartmann) 35.4% (as glucose) and ash 2.51%. The diet was designed to be complete for the rat exclusive of its protein content. This necessitated the addition of a small

[1] Anderson, R. K., and Farmer, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 21.

[2] Anderson, R. K., Fogelson, S. J., and Farmer, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 518.