

and Anatolii(4). This suggests that endotoxin is not involved in production of ascites. Sensitization for production of ascites was transferred passively by lymph node cells but not serum from pertussis sensitized donors. It seems likely, therefore, that the ascites phenomenon is analogous to the cutaneous hypersensitivity reaction of Rowley *et al.*(1) because in both cases there are similar responses to 1 and 2 injections of pertussis vaccine(2) and reactivity can be transferred passively with cells from sensitized donors. Cortisone decreased ascites but it is uncertain whether this was due to immunological effects, aberration of electrolyte and water metabolism, or general debility.

**Summary.** Rats sensitized and challenged intraperitoneally with pertussis vaccine developed severe ascites. However, pertussis vaccine that had been heated for 1 hour at 100°C neither sensitized rats for subsequent challenge nor elicited ascites in adequately

sensitized rats. Unheated pertussis vaccine caused lesions in omentum, lymph nodes and spleen, and excessive gain in weight. These were not observed in animals injected with heated vaccine. Sensitization for production of ascites by pertussis vaccine was transferred passively to normal rats by intraperitoneal injection of lymph node cells but not serum obtained from sensitized rats. The results suggest that ascites elicited by pertussis vaccine resembles the delayed type of hypersensitivity and is not related to endotoxin.

1. Rowley, D. A., Chutkow, J., Attig, C., *J. Exp. Med.*, 1959, v110, 751.
2. Levine, S., Gruenewald, R., in press.
3. Kind, L. S., *J. Immunol.*, 1956, v77, 115.
4. Anatolii, S. A., *J. Microbiol. Epidemiol. Immunobiol.*, 1960, v31, 626.
5. Waksman, B. H., Matoltsy, M., *J. Immunol.*, 1958, v81, 235.

Received July 12, 1961. P.S.E.B.M., 1961, v108.

### Decrease of Sialic Acid in Epiphyseal Plates of Aminoacetonitrile-Treated Rabbits.\* (26861)

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Extensive lesions occur in the connective tissues of animals treated with Lathrogenic agents(1). The epiphyseal plates of young mammals are particularly vulnerable to the action of aminoacetonitriles (AAN). The biochemical nature of the lesion produced with these compounds is poorly understood.

The importance of sialic acid in the connective tissues has been recently emphasized (2,3). The content of sialic acid in the epiphyseal plates of normal and AAN-treated rabbits is here reported.

**Method.** Twenty 8- to 9-day-old rabbits from 3 different litters were divided into 2

groups. One group of 11 animals received a daily subcutaneous injection of 10 mg AAN during 5 days. The AAN solution was prepared dissolving AAN sulfate neutralized with sodium carbonate in saline solution and stored in a refrigerator at 5°C. Nine control animals received no injections. After 5 days the animals were sacrificed by decapitation and the epiphyseal plates from tibia, femur, and distal radius and ulna were obtained and placed in test tubes in ice. The epiphyseal plates were homogenized in a potter in 0.1 N H<sub>2</sub>SO<sub>4</sub> as suggested by Svennerholm(4). The homogenate was hydrolyzed during one hour at 80°C. The hydrolyzate was filtered in a Dowex 50 column and absorbed in Dowex 2 × 8 (analytical grade). The sialic acid was eluted in 1 M acetate buffer pH 4.6 and the eluate was treated with resorcinol copper

\* This work was supported by a research grant from U. S. Public Health Service.

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TABLE I. Sialic Acid in Epiphyseal Plate of Normal and Aminoacetonitrile (AAN-Treated Rabbits).

Normal animals			AAN-treated animals		
Tissue tested, mg (f.w.)	Sialic acid, mg	mg/100 mg (f.w.)	Tissue tested, mg (f.w.)	Sialic acid, mg	mg/100 mg
170.2	120.5	70.7	240	133.5	56.2
209	142.4	68.1	153.5	90.5	58.9
132.7	128.5	96.8	203.2	121.0	59.5
146.0	137.5	94.1	198.3	107	53.9
141.8	123.0	86.7	153.4	136.5	89.2
96.2	90.5	94.0	168.7	128.5	76.1
86.5	75.0	86.7	145.4	128.5	88.3
116.1	86.5	74.5	134.4	90.5	67.3
130.0	153.0	117.0	158.3	126.5	79.9
			190.8	102.5	53.7
			121.3	100.0	82.4
Avg $\pm$ stand. dev.		87.6 $\pm$ 15.28	69.58 $\pm$ 13.93		

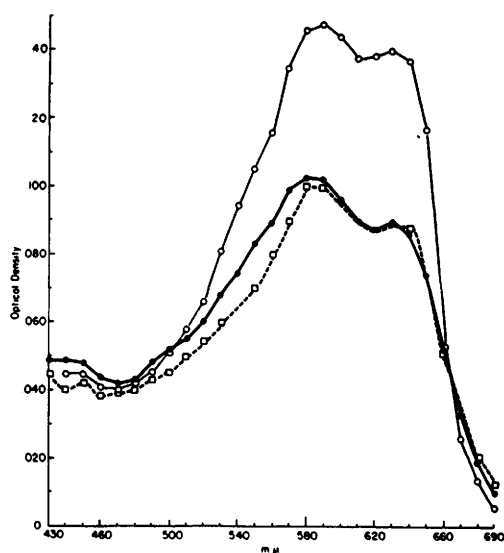


FIG. 1. Absorption curves of chromogens obtained on heating the resorcinol reagent with: Eluate of normal cartilage (mg 146) (—), and of lathyrus cartilage (mg 240) (---) after acid hydrolysis (0.1 N H<sub>2</sub>SO<sub>4</sub>) at 80°C during one hr; standard sialic acid (40 μg) (O....O).

reaction as described previously(2). Optical density was measured with a Beckman's spec-

trophotometer D.U. The values were compared with the standard curve prepared with sialic acid.<sup>§</sup>

*Results* are reported in Table I. A slight decrease in content of sialic acid was observed in the epiphyseal plates of lathyrus animals as compared with the normals.

The comparison between the absorption spectra of sialic acid obtained from the epiphyseal plates of normal and lathyrus rabbits after resorcinol copper reaction is also reported (Fig. 1).

*Summary.* Sialic acid is present in the epiphyseal plate of normal animals and is slightly reduced in the epiphyseal plates of AAN-treated rabbits. No qualitative differences were found in the sialic acid of the epiphyseal plates of normal and AAN-treated rabbits tested.

1. Ponseti, I. V., *Clin. Orthopedics*, 1957, v9, 131.
2. Castellani, A. A., Ferri, G., Bolognani, L., Grazi-  
ano, V., *Nature*, 1960, v37, 185.
3. Anderson, A. J., *Biochem. J.*, 1961, v78, 399.
4. Svennerholm, L., *Acta Chem. Scand.*, 1958, v12, 547.

<sup>§</sup> Kindly supplied by Dr. Winzler.