TABLE I. Glial Response to Various Stimuli Administered on Day of Birth.

Stimulus	Glial diaphorase activity* 5 days after operation TPNH	DPNH	Glial diaphorase activity* 10 days after operation TPNH	DPNH
None	1+	1+	1+	1+
Trocar wound	1+	1+	1+	1+
Paraffin implant	1+	1+	1+	1+
Newborn brain implant	2+	2+	2+	2+
Adult brain implant	4+	3+	4+	3+
Boiled adult brain	4+	3+	4+	3+

* Enzyme activity estimated by formazan deposition after 10 min. incubation: 4+ maximum deposition, deep blue; 3+ to 1+ progressively lighter blue.

while an implant of paraffin is without effect. The mild but definite response to implants of non-viable newborn brain tissue suggests that the inducing agent is present at a lower concentration, but seems at variance with the lack of response to a simple penetrating wound. Other work, however, has demonstrated that very little tissue necrosis results from a penetrating wound of the newborn brain. Therefore the two situations are not comparable, since a relatively large volume of necrotic tissue is present following implantation of newborn brain tissue. It would seem that the postulated inducing agent is present in newborn brain tissue, and that the failure to incite reactive gliosis may in part be a consequence of failure to liberate the inducer from inactive tissue compartments *via* the mechanism of necrosis.

It is of interest that prior boiling of implanted adult brain tissue does not lessen the inductive effect. The heat stability of the inducing factor excludes certain heat labile compounds in the search for the effective agent, and also excludes the possibility that the reactive astrocytes observed originate from within the implant.

Conclusion. This study clearly demonstrates that astrocytes of newborn rat brain display the morphologic and enzymatic changes of reactive gliosis in response to the appropriate stimulus. The absence of newborn glial response to simple penetrating wounds may be a result of ineffective liberation of an inducing agent present at low concentration.

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Hexosamine-Synthesizing Enzyme in Human Arterial Tissue.* (29880)

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The hexosamine-synthesizing enzyme which was first discovered by Leloir and Cardini(1) in *Neurospora crassa* has subsequently been demonstrated in some mammalian tissues, whereas several tissues do not display any activity(2,3). The presence of a clearly measurable hexosamine synthetase activity in the aorta of young rabbits was observed by Bolognani *et al*(4). This enzyme is an aminotransferase which catalyzes the following reaction: Glucose-6-phosphate + L-Glutamine ⇄ Glucosamine-6-phosphate + Glutamic acid.

Because this enzyme is involved in the biosynthesis of mucopolysaccharides it was considered of importance to study its activity in human arterial tissue. For comparative purposes, a limited number of venous samples was included in the investigation. In addition, enzyme assays were made separately on normal and arteriosclerotic arterial tissue.

Materials and methods. Vascular samples

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TABLE I. Hexosamine Synthetase Activities of Human Blood Vessels.*

	No.	Mean hexosamine synthetase activity: Per g wet tissue	S.E.M.	Per g tissue nitrogen	S.E.M.
Thoracic descending aorta:					
Normal	41	.606	.030	15.18	.78
Arteriosclerotic (lipid)	26	.588	.042	17.04	1.38
" (fibrous)	5	.636	.120	17.58	3.36
Ascending aorta, normal	2	.720	—	17.46	—
Abdominal aorta:					
Normal	5	.726	.012	18.60	1.56
Arteriosclerotic (lipid)	2	.828	—	20.22	—
Pulmonary artery, normal	20	.450	.030	12.78	.90
Coronary artery:					
Normal	12	.450	.036	13.62	1.32
Arteriosclerotic (lipid)	5	.390	.096	14.16	4.02
Cerebral arteries, normal	2	.372	—	12.36	—
Vena cava inferior	5	.618	.102	16.50	2.28

* Values expressed in mg of hexosamine formed per gram of wet tissue and per gram of tissue nitrogen in 20 min.

were obtained at autopsy from subjects ranging in age from 1 to 75 years and were immediately frozen. A total of 125 specimens were included in the study. The hexosamine synthetase measurements were made by a modification of the method described by Bolognani *et al*(4).

The assay solution consisted of 0.01 M glucose-6-phosphate, 0.01 M L-glutamine, and 0.067 M phosphate buffer, pH 7.4. Since it has been established(3) that glucose-6-phosphate protects the hexosamine-synthesizing enzyme, 1.5% homogenates of intima-media layers of the vascular samples were prepared in the assay medium. Homogenization was performed at a temperature of 4°C with a Kontes Duall type tissue grinder.

The hexosamine formed in the presence of substrate and enzyme was determined before and after 20 minutes' incubation in a shaking water bath at 30°C. At zero time and at the end of the incubation period, a 3.0 ml aliquot was removed from the incubation media and transferred to a test tube. The enzymic activity of the aliquot was immediately stopped by addition of .75 ml 1.8 N HCl. The acid-treated samples were placed in a boiling water bath for 10 minutes; the tubes were tightly closed to prevent change in sample volume. After cooling, the tubes were centrifuged for 5 minutes at 2500 r.p.m. One ml of the resulting clear supernatant was used for hexosamine determination by the method of Schloss(5), the optical density readings being made with a Beckman DU spectrophotometer. All hexosamine determinations were made in duplicate. No color formation by the Schloss procedure was observed in assays of substrate reagent blanks which had been incubated in the water bath simultaneously with the tissue test samples. Tissue samples pretreated with 1.8 N HCl did not reveal any increase in hexosamine content following incubation in a water bath at 30°C.

Experiments showed that the hexosamine formation during the first 20 or 30 minutes closely follows a parabolic curve; when the amount of synthesized hexosamine is plotted against the square root of time of incubation, a straight line is obtained. Hexosamine synthesis by arterial tissue was found to be very low after a 30-minute incubation period; a 20-minute period was therefore used for assays. The results are expressed in milligrams of hexosamine formed per gram tissue in 20 minutes. A similar enzymatic behavior was observed for the synthetase present in young guinea pig aortas.

Results. The average hexosamine synthetase activities determined for various types of blood vessels are listed in Table I. Approximately similar values were recorded for

aortic tissue and for the inferior vena cava. A tendency toward lower hexosamine synthesis was observed for the pulmonary artery and coronary artery samples. Assays performed on normal and lipid-arteriosclerotic aortic tissue specimens showed no significant difference between mean enzymatic activities exhibited by these tissue portions.

Discussion. The hexosamine-synthesizing enzyme has recently received increasing attention because this aminotransferase is involved in the synthesis of mucopolysaccharides. No investigations have previously been reported on the presence of this enzyme in human vascular tissue. The finding that one gram of human aortic tissue is capable of synthesizing .6 mg hexosamine in 20 minutes suggests an appreciable activity of this synthetase in the aortic wall. Since it has been suggested by various investigators that the mucopolysaccharides in the arterial wall may be closely connected with the pathogenesis of arteriosclerosis, quantitative biochemical studies of the various enzyme systems in arterial tissue associated with the intermediary metabolism of mucopolysaccharides may prove to be of definite significance in evaluating disease processes.

Summary. Determinations were made of the hexosamine-synthetase activities exhibited by intima-media layers of various types of human blood vessels. A total of 125 vascular specimens was included in the study. The activity measurements were performed by a modification of the procedure described by Bolognani *et al.* The average quantity of hexosamine synthesized in 20 minutes by one gram of thoracic descending aorta was .606 mg; corresponding values for the ascending aorta, abdominal aorta, pulmonary artery, coronary artery, cerebral arteries, and inferior vena cava were, respectively, .720, .726, .450, .450, .372, and .618. No significant difference was observed between the activity of normal and arteriosclerotic tissue from the same arterial specimens.

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Effect of Sham Feeding on Bile Flow in Cholecystectomized Dogs. (29881)

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Insulin hypoglycemia has been shown(1) to produce a choleresis in chronic cholecystectomized dogs when gastric acid secretion was excluded from the duodenum. This action was blocked by a continuous intravenous infusion of an anticholinergic drug and by bilateral vagotomy. Feeding also elicited a choleresis which did not occur after vagotomy. These results were consistent with a vagally-mediated action on the liver. To obtain further evidence for the role of the vagus in choleresis and to detect a "cephalic" phase of bile secretion, bile flow was determined in the conscious cholecystectomized dog after sham feeding.

Method. The studies were carried out on 3 mongrel dogs weighing between 12-17 kg. Each dog was prepared surgically by cholecystectomy, ligation of the lesser pancreatic duct, installation of gastric and duodenal cannulae(2) and a 2-stage esophagostomy(3). The duodenal cannula was so placed that on opening it the ampulla of the common bile duct was visible. The experiments were car-