differences between the 2 species indicate that interarterial coronary anastomoses in hearts of neonates are not a general characteristic of the mammalian phylum but rather of man. Whether they are unique to the latter requires the study of neonatal hearts in other species.

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Allergic Reactions to Hyaluronidase.* (26458)

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During the past several years a number of reports of allergic reactions to hyaluronidase have been published(1-5). Holborrow and Keech(1) believed that the occurrence of hypersensitivity reactions in their patients was probably due to presence of protein impurities in the hyaluronidase preparations employed. Although the purity of more recent preparations has been emphasized(2), the data to follow will indicate that a highly purified commercial hyaluronidase extracted from bull testes was found to contain considerable bovine serum proteins. Such impurities could easily have been responsible for the reported allergic reactions to hyaluronidase.

Experimental. An adult albino rabbit was injected subcutaneously once or twice weekly with 1-4 mg of hyaluronidase (Wydase)† in Freund's adjuvant. A total of 16 mg was administered over 5 weeks. Five days after the last injection the animal was bled and its serum tested for antibodies to bovine serum albumin (BSA) and bovine gamma globulin (BGG) by the hemagglutination technic of Stavitsky and Arquilla(6). BSA or BGG coupled to rabbit red blood cells with bisdiazotized benzidine served as antigens.

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† Wyeth Laboratories, Philadelphia, Pa.

Table I shows that the serum of the rabbit injected with hyaluronidase had a titer of 1:10,240 to BSA and a titer of 1:10,240 to BGG. Pre-incubation of this serum with 1 mg of hyaluronidase reduced the titer to zero. In the next experiment a series of dilutions of a rabbit anti-BSA serum (titer 1:25,600) was incubated with various amounts of BSA or hyaluronidase prior to addition of BSA coupled to red cells. Table II demonstrates that 1 mg of hyaluronidase reduced the anti-BSA serum titer to zero and 0.1 mg lowered the titer to 3200; .01 mg of hyaluronidase had no inhibitory effect. Comparison of the above inhibition with that produced by BSA (Table II) suggests that the hyaluronidase preparation employed had 1/100 to 1/1000 the inhibiting activity of BSA. Pre-incubation of rabbit anti-rye grass serum with 1 mg of hyaluronidase did not lower the titer of this serum to rye grass pollen (Table II).

In a third experiment mice were sensitized to BSA by intraperitoneal injection of 5 billion *Bordetella pertussis* organisms and 4 mg of BSA(7). Ten days later the animals were challenged intravenously with various amounts of BSA or hyaluronidase. Due to the small amounts of material used in challenge, fatal anaphylaxis did not take place

TABLE I. Demonstration of Antibodies to Bovine Serum Proteins in Serum of a Rabbit Injected with Hyaluronidase.

Rabbit antiserum	Inhibiting antigen*	Antigen on red cell	Reciprocal of titer
Anti-hyaluronidase	—	BSA†	10,240
"	—	BGG‡	10,240
"	1 mg hyaluronidase	BSA	0

* Incubated with antiserum for 30 min. at room temperature prior to addition of antigen on red cell.
† Bovine serum albumin.
‡ Bovine gamma globulin.

(ordinarily 1 mg of BSA will kill 50-75% of the animals). The occurrence of anaphylaxis was determined by noting symptoms and recording the fall in rectal temperature after challenge(8,9). The data in Table III demonstrate that both BSA and hyaluronidase caused symptoms of anaphylaxis and a fall in rectal temperature in BSA sensitive mice. The activity of the hyaluronidase preparation was approximately 1/200 to 1/1000 that of the BSA, a ratio very similar to that obtained by the hemagglutination inhibition technic (Table II). There seems little doubt that our hyaluronidase preparation contained bovine serum albumin (0.1-1%), bovine gamma globulin, and other serum components, although it is conceivable that the results obtained were due to the fact that bovine hyaluronidase has antigenic determinants similar to those in BSA or BGG. In view of the probability that other commercial hyaluronidase preparations also contain bovine serum proteins as impurities, it may be advisable to skin test a patient with bovine serum prior to administration of hyaluronidase.

TABLE II. Inhibition of Hemagglutination by BSA and Hyaluronidase.

Rabbit antiserum	Inhibiting antigen	Antigen on red cell	Reciprocal of titer
Anti-BSA	—	BSA	25,600
"	1 mg hyaluronidase	"	0
"	.1 " "	"	3200
"	.01 " "	"	25,600
"	10 μg BSA	"	0
"	1 " "	"	400
"	.1 " "	"	3200
Anti-rye grass	—	Rye grass	25,600
Idem	1 mg hyaluronidase	*Idem*	25,600

TABLE III. Anaphylaxis in BSA Sensitive Mice after Challenge with BSA or Hyaluronidase.

Mice sensitized with	Challenged 10 days later with	Reactors* /Total
—	25 μg BSA	0/10
.1 ml pertussis vaccine + 4 mg BSA	5 " "	1/8
Idem	25 " "	6/8
"	5 mg hyaluronidase	5/10
"	2.5 " "	0/7
—	5 " "	0/10

* Symptoms of anaphylaxis plus a temperature drop greater than 2°C.

Summary and conclusions. It has been demonstrated by hemagglutination inhibition tests as well as by induction of anaphylactic shock that a commercial hyaluronidase preparation contained a small but appreciable amount of bovine serum proteins. In view of the known allergenic properties of such impurities it is suggested that skin tests with bovine serum precede administration of hyaluronidase.

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