sponses could be detected in children yielding type 16 isolates, there were abundant data to indicate that the children themselves were the source of these viruses and not the tissue culture system. For example, in the routine 1958-59 testing type 16 isolates were obtained only from anal specimens despite the fact that both throat and anal specimens were tested simultaneously in the same tissue cultures.

Because of the frequency with which undifferentiated illnesses occur in the Junior Village population and the infrequent testing for the little-known adenoviruses, no information was obtained on the relationship of these agents to the common illnesses in the population.

Summary. The occurrence of a number of little-known adenovirus serotypes in children in an institution in Washington, D. C., is described. These viruses, which included types 9, 10, 12, 13, 16, 17, 24, 25, 26, 27, 28, BP-6, and BP-7 were isolated almost exclusively from anal specimens. As a group they were almost as common as the better known serotypes 1, 2, 3, and 5. It was shown that the latter types, although frequently present in routine throat specimens, were also more frequently isolated from routine anal specimens. Homotypic hemagglutination-inhibition antibody rises were demonstrated in children infected with most of the little-known serotypes.

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Source and Time of Release of Histamine-Destroying Factor (Histaminase) During Anaphylactic Shock in Rats.* (27736)

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Code and associates(1) have recently demonstrated the release of a histamine-destroying factor during anaphylactic shock in albino rats. This factor was released during shock whether the rats were sensitized using pertussis vaccine as an adjuvant or were adrenalectomized prior to sensitization without an adjuvant. The amount released in adrenalectomized rats was less than in intact rats. The factor has been tentatively identified as histaminase.

Waton(2) has shown that most of the histaminase in normal rats is found in the duodenum, ileum, and colon. Some of the enzyme, however, may be present in the stomach, liver, kidney, and lung. Lindell and Westling(3) have demonstrated that in the guinea pig the kidney and intestinal tract contain only small amounts of histaminase whereas the liver is rich in the enzyme. Our study was undertaken to determine the source of the histaminase released into the blood stream of the rat during anaphylaxis.

Method. White rats, mostly males of the Sprague-Dawley strain, weighing 150 to 260 g were used. The intact animals were sensitized by a single intraperitoneal injection of

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TABLE I. Blood Histamine of Sensitized Rats after Shock: Related to Time of Withdrawal of Blood.

Animal	Min. between inj. of antigen and withdrawal of blood*	Histamine in blood: After immediate extraction, μg/ml	Histamine in blood: % destruction after incubation† followed by extraction
1	2½- 4	1.3	0
2	3 - 4½	2.0	15
3	3½- 4½	.9	0
4	4 - 4½	.5	0
5	4 - 8	1.2	91
6	6 - 7	2.0	45
7	6 - 8	1.5	86
8	6 - 8	.64	86
9	6 - 8	1.5	91
10	6 -11	1.4	67

* The 2 figures refer to time that withdrawal began and time it was terminated.
† Incubated for 1½ hr at 37°C.

1 ml of undiluted horse serum combined in the same syringe with 0.5 ml of a saline suspension of phase 1 Bordetella pertussis vaccine (Lederle) containing approximately 30 $\times$ 10^9 killed pertussis organisms. Most of the animals were challenged with 0.2 ml of undiluted horse serum given intravenously 13 or 14 days after the sensitizing dose. Two were challenged with 0.9% saline solution instead of the horse serum (sham shock).

Anesthesia was induced by intraperitoneal injection of 3.5 to 4 mg of sodium pentobarbital per 100 g of body weight in a 25% solution.

A No. 60 polyethylene catheter was inserted into the right atrium *via* the external jugular vein. In a few instances the catheter reached the upper portion of the inferior vena cava or the lower part of the superior vena cava. Following this procedure laparotomy was performed through a long right rectus incision. No laparotomy was carried out in the animals used only for the time of release studies (Table I). In most instances animals were operated in groups of 4: In one of the 4 animals both renal and intestinal vessels† were ligated, in the second only the intestinal vessels, and in the third only the renal vessels. In the fourth the ligatures were placed in position but not tied (sham operation). After the ligatures had been placed, the abdomen was closed with running suture.

A control sample of blood was then withdrawn through the previously inserted catheter. The challenge dose of horse serum was then injected intravenously. Blood was withdrawn for determination of histamine 2 to 5 and 6 to 13 minutes after challenge. The control specimen of blood and approximately half of the specimen drawn after shock were subjected immediately to the extraction procedure with trichloracetic acid (4). The other half of the "shock" specimen was incubated at 37.5°C for 1½ hours in a constant-temperature water bath. Following this, it also was subjected to the extraction procedure. All extracts were subsequently assayed on the isolated atropinized guinea pig ileum. The results are expressed as the amount of histamine base in 1 ml of blood.

After withdrawal of the shock specimens of blood, 2 ml of India ink were injected intravenously into each rat in which a vessel had been ligated. The adequacy of vessel ligation and proper positioning of the plastic catheter were checked. In those animals in which the intestinal vessels were tied, the blood supply of the gastrointestinal tract from the antrum of the stomach to the midcolon was excluded from the circulation.

Results. Control samples of blood were withdrawn from 40 rats immediately after the ligation procedures. In 30 the values of histamine ranged from 0.03 to 0.09, and in 10, from 0.1 to 0.15 μg of histamine per ml of blood. The mean of the 40 values was 0.082 $\pm$ 0.005 μg/ml.

In the 2 sensitized rats subjected to ligation that received 0.2 ml of 0.9% saline instead of horse serum as the shocking dose, the value of the blood histamine did not vary from the control value when blood was withdrawn 5 minutes after the saline solution had been injected.

Preliminary studies on 32 rats disclosed that a considerable quantity of histamine was released into the systemic circulation following shock in all animals whether the intestinal vessels or renal vessels or both were ligated. Significant destruction of histamine after incubation, however, was observed after shock

† Intestinal vessels consisted of the superior mesenteric artery, celiac axis and portal vein.

TABLE II. Blood Histamine* of Sensitized Rats 6 to 13 Minutes after Shock. (Each pair of values from one rat.)

	Vessels ligated							
	Intestinal and renal		Intestinal		Renal		Sham operation	
Group	Immed. extract.	Incubation† followed by extract.	Immed. extract.	Incubation† followed by extract.	Immed. extract.	Incubation† followed by extract.	Immed. extract.	Incubation† followed by extract.
1	2.0	2.57	1.85	1.45	.8	.28	1.3	.21
2	.56	.48	.52	.40	.28	.26	2.2	.32
3	2.0	1.8	3.75	1.5	.36	.06	—	—
4	—	—	.16	.16	1.2	.09	1.4	.18
5	2.6	2.6	1.1	1.0	.7	.25	1.2	.6
6	.4	.33	.8	.8	.6	.43	—	—

* Expressed as μg of histamine per ml of whole blood. † Incubated for 1½ hr at 37°C.
— = Failure of rat to survive long enough to obtain specimen.

in only a few of those animals in which renal vessels were ligated or in which a sham operation had been performed. No destruction was observed in those in which the intestinal vessels were ligated.

A group of 10 sensitized rats was then challenged without previous laparotomy. Blood was withdrawn at varying times after the shocking dose was administered intravenously. The results are shown in Table I. No significant destruction of histamine was noted in the blood from any animal when the withdrawal was complete 4½ minutes or less after injection of the antigen. Whenever withdrawal was not complete in less than 7 minutes after injection of antigen, significant destruction of histamine was observed after incubation. A review of the protocols of the preliminary studies showed that in most instances withdrawal of blood had been completed within 4½ minutes after the shocking dose of antigen had been administered.

Twenty-one rats were subjected to ligation and shock. Samples of blood were withdrawn 6 to 13 minutes after the antigen was given. The results are given in Table II.

No destruction of histamine was observed in the blood from animals in which both intestinal and renal vessels were tied. Destruction was observed, however, in one of the 6 in which the intestinal vessels only were tied, and in 5 of the 6 in which renal vessels only were tied. In all 4 animals subjected to the sham operation, there was evidence of histaminase release after shock.

Comment. The mean value for histamine of 0.082 ± 0.005 μg/ml in blood withdrawn after the ligation procedures had been completed is almost identical with the value found by Code and associates(1) for normal intact rats (0.078 mg/ml). Thus the operative procedure did not alter blood histamine values. No elevation of histamine values was observed in a period of 5 minutes after injection of saline solution instead of antigen (sham shock). These studies indicate that ligation of the intestinal and renal vessels does not produce a delayed increase in concentration of histamine in the blood.

The release of histaminase during anaphylactic shock in rats observed in this study confirms the prior observation of others(1). The time lag (Table I) between injection of the antigen and appearance of histaminase in the blood has not been appreciated previously. Although in some instances significant amounts may be found less than 6 minutes after injection of the antigen, it seems to be noted with more consistency only after this time. This is in contrast to its prompt appearance in the guinea pig in anaphylactic shock(5).

The data in Tables I and II do not support the consideration that there is a critical level of blood histamine above which histaminase is released. It is possible that such a critical level could exist in the tissue spaces. If this is so, histaminase must be produced in a limited quantity and utilized at or near the site of production.

The failure to find any evidence of significant destruction of histamine following incu-

bation of the blood of rats in which both intestinal and renal vessels had been ligated regardless of the time interval after injection of the antigen is evidence that the enzyme is released from one of these 2 areas. With one exception the animals with ligated renal vessels showed evidence of histaminase release when the blood was drawn 6 to 13 minutes after injection of the antigen. Hence, it must be concluded that little if any histaminase is released from the kidneys following anaphylactic shock.

With one exception the data in Table II strongly favor release of histaminase only from the intestinal tract. This confirms the *in vitro* findings of Waton(2) in a study carried out in the tissue of normal animals.

The source of histamine released during anaphylactic shock in the rat is still not clear. The perfusion studies of Brocklehurst (6) indicate that histamine is not released from lung, liver, or hindquarters. Our study shows that histamine is released despite ligation of both intestinal and renal vessels. This seems to eliminate the small intestine which has previously been considered the source(1, 7).

Conclusions. 1. Histaminase is released after anaphylactic shock in rats. Its release is consistently noted in blood drawn 6 to 13 minutes after injection of the antigen, less consistently in blood drawn prior to 6 minutes. 2. The site of histaminase release during anaphylactic shock in rats is the intestinal tract, probably the small intestine. 3. Histamine is released into the blood stream following anaphylactic shock in the rat despite ligation of intestinal and renal blood vessels.

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Hepatic Antigens in the Blood of Rats with Toxic Liver Damage.* (27737)

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Liver cell disintegration and the increase of enzymes in plasma during acute hepatic damage(1,2,3) suggest leakage of liver substances into the blood. Furthermore, release of hepatic proteins into blood perfusing isolated rat livers has been shown by Ouchterlony's method(4). We present here evidence obtained by the double diffusion technic(5) for the presence of hepatic antigens in the blood of rats with toxic liver injury, and description of the time course of the levels of these antigens and their immuno-electrophoretic(6) behavior.

Methods. Anti-rat liver serum. Antiserum was prepared in 10 rabbits according to Freund's technic(7), employing whole rat liver homogenate as the antigen. Sera showing the highest levels of antibodies were pooled and merthiolate was added to a final concentration of 1 × 10,000. Anti-rat serum protein antibodies were absorbed by successive addition of normal rat sera until no pre-

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