Cobayin: A Poisonous Principle in the Guinea Pig's Seminal Plasma.*†‡ (29730)

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To our knowledge, poisonous substances have not been found previously in secretions of warm blooded animals. We were, therefore, surprised to observe that the seminal plasma of guinea pigs is highly toxic to rabbits on parenteral introduction. This finding is in complete contrast to our considerable experience with the seminal plasmas of man and rabbit(1,2), which are well tolerated when injected into rabbits, guinea pigs, mice and chickens.

This peculiarity of the guinea pig's seminal plasma was observed when we started injecting rabbits for the purpose of obtaining anti-guinea pig seminal plasma immune sera. This report presents data collected in following up this observation.

Methods. Seminal plasma of guinea pigs was obtained in the form of the supernatant fluid of semen collected by the electroejaculation method of Freund(3). The small amount of seminal plasma obtainable from the individual ejaculate (about 0.3 ml) is an obvious limiting factor for experimentation. Six or more specimens were pooled for use in the experiments described below. When feasible, fresh material was used. However, no difference in properties was noted when specimens were employed that had been kept frozen at —20°C. Before injection, the seminal plasma was diluted in physiological saline solution as indicated below.

TABLE I. Lethal Effect of Guinea Pig Seminal Plasma Injected into Rabbits.

Dose, ml	Route of injection		
	Intrav	Intraper	Subcut
.2	5/6	6/10	4/7
.02	5/32	5/10	1/4
.002	6/9	1/4	
.0002	0/4		

Numerator = No. of animals dead after 20 hr; denominator = No. of animals injected.

The rabbits used were of mixed breed and weighed 2.3-2.9 kg. Mice were of the CF-1 strain and of 25-28 g weight. Rats of the Wistar strain weighed ca. 200 g. Guinea pigs were of mixed breed and weighed about 500 g. Dogs were mongrels of weights given below.

Results. As shown in Table I, 81 rabbits were injected by various routes with guinea pig seminal plasma in doses between 0.2 and 0.0002 ml diluted to a volume of 2.0 ml. Within minutes after injection of seminal plasma, the animals showed agitation and flushing of the ears, followed by labored breathing, diarrhea and weakness. The animals died one-half to twenty hours after injection. There was no striking difference in symptoms and time of death between rabbits that received as much as 0.2 ml and as little as 0.002 ml of seminal plasma. On autopsy, no gross pathological changes were seen. Usually, but not always, the right ventricle and the atria of the heart were distended with clotted blood, while the left ventricle was contracted and nearly empty. Microscopic study of the tissues is under way.

About half of the animals that survived after intravenous or intraperitoneal injection showed signs of severe intoxication for 2 to 4 hours. They showed no signs of illness after

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† After this work was essentially completed, Dr. Seymour Katsh (Univ. of Colorado) told us, that he had observed toxicity of guinea pig seminal plasma in rabbits. This observation had not been followed up (Personal communication).

‡ After this report had been submitted for publication, it came to our attention that J. Freund and G. E. Thompson (PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 350) described toxic effects produced by injection of fluid from the coagulating gland of the guinea pig into rabbits and guinea pigs. These findings differ from those noted here in several important points, such as the toxicity for the guinea pig itself. The relationship of cobayin to the observations of Freund is now being investigated.

TABLE II. Effect of Guinea Pig Seminal Plasma on Injection into Rats and Mice.

	Dose, ml	No symptoms	Sick	Died	Deaths/Total
A. Rats, intrav inj	.5	0	1	3	3/4
	.1	1	15	2	2/18
B. Rats, intraper inj	.1	5	3	6	6/14
	.02	0	5	0	0/5
C. Mice, intrav inj	.1	10	0	5	5/15
	.01	6	0	0	0/6
D. Mice, intraper inj	.1	5	5	0	0/10
	.01	0	2	0	0/10

that period and no visible pathological changes when sacrificed after 4 to 7 days.

We are indebted to Dr. F. Wilcoxon for a calculation of the LD_{50} for the intravenously injected rabbits according to the method of Bliss(4). It gave a value of 0.0012 ml with upper and lower 95% confidence limits of 0.0100 and 0.00015 ml. These wide limits are partially explained by the rather flat slope of regression. Also, a relatively large number of the animals received doses of 0.02 ml, due to the fact that more than half of these animals had been serving as toxicity controls in the experiments reported in the following paragraphs. Toxicity upon intraperitoneal application is less than that by intravenous route by about one order of magnitude, and less than that by subcutaneous injection by about two.

Feeding by gavage of 0.5 ml seminal plasma to 4 rabbits did not affect the animals.

White rats, white mice and dogs are also susceptible to the toxic effect of guinea pig seminal plasma, though notably less so than rabbits. The rats were injected with seminal plasma diluted to 0.5 to 1.0 ml. The mice received the plasma diluted to 0.5 ml for intraperitoneal and 0.25 ml for intravenous injection. Results are shown in Table II. Animals designated as "sick" showed severe cyanosis, labored or spasmodic breathing, great weakness, often to the point that they could not maintain an upright position. They recovered in 3 to 4 hours without sequelae. Those that did not recover, died in 3 to 20 hours. On autopsy, some showed hyperemia of the intestines or patchy hyperemia of the lungs. No other gross changes were noted.

Of 3 dogs injected with guinea pig seminal plasma, one, weighing 19 lb, received 0.2 ml diluted to 2.0 ml subcutaneously. For about 2 hours, he retched and vomited many times and trembled violently. During the next hour, he had several diarrheic stools, but was more lively than before. After 4 hours, he had recovered. The second dog (24 lb) received the same dose intravenously, showed the same signs, but to a much more severe degree. Dejecta and vomit became bloody. There was extreme weakness, salivation and labored breathing. He expired in 7 hours. The third dog (37.2 lb) injected with the same dose intravenously, presented the same clinical signs for about 6 hours, after which period he slowly improved. In about 20 hours he was completely recovered.

The guinea pig itself is not susceptible to the agent in doses of 0.2 ml as shown by the lack of signs of intoxication in 12 animals (500 g weight) injected by the intracardial or intraperitoneal route.

The toxic material does not pass the wall of Visking tubing (ca. 50 Å pore size). After 24 hours dialysis, the material inside the tube had not lost toxicity and the outside fluid did not cause any toxic effect in an amount equivalent to 0.2 ml of the original seminal plasma.

Undiluted or diluted guinea pig seminal plasma coagulates at temperatures of 65°C and above. However, when seminal plasma diluted 1/10 in physiological saline solution is passed through a 10 mμ Millipore filter, kept at a temperature of 95-98°C for 10 minutes, and the resulting precipitate removed by refiltering through the same type of filter, the filtrate has the characteristic lethal effect in a dose corresponding to 0.02 ml of the

original seminal plasma. It would, therefore, appear that the toxic principle is heat resistant to a considerable extent.

Exposure to 0.4% formaldehyde for 48 hours (followed by dialysis) did not diminish toxicity noticeably. However, a concentration of 2% formaldehyde yielded a preparation which was not toxic to rabbits at a level of 0.02 ml in terms of original seminal plasma.

Antisera against guinea pig seminal plasma was obtained in rabbits, mice, and rats injected with sublethal doses. Seminal plasma of man and other animals is known to contain more than one antigenic component, especially spermatazoa-coating antigen (SCA) (1,2,5). At present, we cannot say whether any of the antibodies of anti-guinea pig seminal plasma immune sera are directed against the toxic material.

Discussion. For the toxic principle described here, the designation cobayin (from the species name *Cobaya* of the guinea pig) is suggested. At least for the rabbit, it must be called "extremely toxic," if the nomenclature suggested by Spector(6) for poisons is applied.

In this respect, it must be considered that the LD_{50} (intravenous) of 0.001 to 0.002 ml refers to rabbits of 2.6 kg average weight. This corresponds to a dose of 0.00035 to 0.0007 ml per kg. We found that guinea pig seminal plasma (pooled) contained 11.3% dry matter. Of this, 6.5% (in terms of the original seminal plasma) is non-dialyzable, all or nearly all of which is protein. The specimens used for these determinations contained 6.3% protein as determined by the biuret method. (In other specimens protein content varied between 8.7 and 4.6%; average = 6.5%.) If roughly 1/15 of the seminal plasma is non-dialyzable matter, 0.023 to 0.045 mg of this represents one LD_{50}/kg rabbit (intravenous). As mentioned before, the LD_{50} on intraperitoneal and subcutaneous application is larger by one and two orders of magnitude respectively. It would be reasonable to expect that only a fraction, and perhaps only a small fraction of the amounts quoted, is actually cobayin. For comparison, it may be mentioned that the lethal dose of crude snake poisons (dry weight) is in the order of milligrams, varying considerably according to species of snake and recipient and route of application(6,7).

We are attempting to separate cobayin from the other constituents of the guinea pig's seminal plasma to obtain information about its chemical nature, its origin, and its antigenicity.

Summary. Guinea pig seminal plasma is highly toxic on parenteral application for the rabbit and, to a lesser degree, for the mouse, rat and dog, but not for the guinea pig itself. The name cobayin is suggested for the toxic principle. It is non-dialyzable, shows considerable resistance to heat, and is detoxified by formalin.

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