
Attempts to Induce Anaphylaxis in the Hamster

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The recognition that reactivation of herpes simplex virus can be accomplished by induction of anaphylaxis or histamine shock in the rabbit (Good and Campbell, 1945, 1948) suggested the possibility of producing the same phenomenon in the hamster previously inoculated with this virus. It was soon observed, however, that small doses of antigen, as employed for the induction of sensitivity in the guinea pig, were without any detectable effect on the hamster. Consequently, it became necessary to study this phenomenon further by determining the response of the animal to larger doses of antigen and observing its reaction to systemic injection of histamine and serotonin. The present communication is a preliminary report of the results of these investigations.

MATERIALS AND METHODS

Animals — Golden hamsters (*Mesocricetus auratus*) were used throughout these experiments.

Sensitization procedures — The animals were sensitized by injecting them intraperitoneally with 2 mg ovalbumin (2× crystallized, Mann Research Laboratories) in saline and, after 14 days, they were challenged with 100 mg of the same material intraperitoneally or 60 mg intracardially

(Campbell et al., 1964). Failure to induce anaphylaxis by this method led to the administration of larger doses of antigen in three successive injections given on alternate days. The challenge dose of antigen was administered 14 days after the last sensitizing injection. A protocol of the procedures employed in these experiments is given in Table I.

TABLE I. SENSITIZATION AND CHALLENGE OF HAMSTERS WITH OVALBUMIN

Animal	Sensitization		Challenge	
	Total Dose (mg)	Mode	Dose (mg)	Mode
1	30	s.c., i.p., i.m.	100	i.p.
2	30	s.c., i.p., i.m.	60	i. card.
3	60	s.c., i.p., i.m.	100	i.p.
4	60	s.c., i.p., i.m.	60	i. card.
5	90	s.c., i.p., i.m.	100	i.p.
6	90	s.c., i.p., i.m.	60	i. card.
7	120	s.c., i.p., i.m.	100	i.p.
8	120	s.c., i.p., i.m.	60	i. card.

Precipitation Tests — The Ouchterlony double diffusion technic was used for detecting precipitating antibody. Results were recorded after 96 hr of incubation at 37 C.

Histamine Shock. — The effect of histamine on the hamster was determined by injecting varying amounts of histamine phosphate (Eli Lilly) intracardially into 3 animals. The first of these received 0.165 mg; the second, 0.495 mg; and the third, 1.10 mg. The injections were performed without anesthesia. A fourth animal was injected with 0.275 mg by the same route, but under ether anesthesia. Blood was drawn from the heart prior to, and three minutes after, the administration of the drug. With each of these samples leukocyte counts, complement titrations, and blood coagulation time determinations were made.

For leukocyte counts, the blood was drawn into a Unopette disposable blood diluting pipette (Becton, Dickenson and Co.) and counts were carried out with a Spencer Neubauer counting chamber. Complement levels were determined by conventional complement titration procedures using 2 units of hemolysin and a 2% sheep cell suspension. The coagulation time was determined by depositing blood in silicone-coated test tubes (Biggs and Macfarlane, 1957) and observing the time required for the formation of fibrin.

RESULTS

Anaphylactic sensitization could not be induced in the hamster by the injection of up to 120 mg of ovalbumin. Mild symptoms which followed the challenge dose, such as scratching, rubbing of the nose, voiding urine, ruffled fur, and general weakness, appeared only irregularly regardless of the dosage of antigen used for sensitization and for challenge and may well have been a part of the normal behavior of the animals under stress. Furthermore, there were no anatomical findings at autopsy to indicate that the symptoms were the result of abnormal physiological behavior arising from previous sensitization.

Weak bands of precipitation were present in Ouchterlony plates with five samples of sera when they were diffused against the antigen.

Injection of histamine phosphate in a dose of 0.165 mg produced no apparent reaction in the hamster. However, when the dose was increased to 0.495 mg, the animal went into shock but recovered after 20 min. Increasing the dose to 1.10 mg caused immediate death of the animal. At autopsy the heart was dilated, possibly due to the amount of fluid injected. The lungs, kidneys, and adrenals were normal in size and appearance but there was a suggestive distention and congestion of the liver.

Before the administration of histamine, the leukocyte count of the test animal was 5,500/mm³, the complement titer was 1:14, and the blood coagulation time was 2½ min. Three minutes after the administration of histamine the values remained essentially unchanged, except for a slight decrease in the leukocyte count (5,100/mm³).

In later work toxic doses of histamine phosphate and serotonin, leukocyte counts, complement titers and blood coagulation times in hamsters were determined under ether anesthesia. It was found that the lethal dose of histamine phosphate inoculated intracardially was 0.825 mg or over, and that of serotonin was extremely variable, ranging from 10 to 30 mg. Likewise, the leukocyte counts varied from one animal to the other, ranging from 1,800 to 9,200/mm³; the complement titers ranged from <1:4 to 1:7.34, and the blood coagulation times varied from 1 to 3 min. Nevertheless, as in previous experiments, following the administration of sublethal doses of histamine phosphate and/or serotonin, these values remained essentially unchanged.

DISCUSSION

Anaphylaxis has been demonstrated in the guinea pig, rabbit, dog, mouse, rat, and other animals (Seegal, 1935; Sherwood, 1951), but there are no reports to indicate that this phenomenon can be induced in the hamster. In the present experiments the administration of up to 120 mg of ovalbumin failed to sensitize the animal. Subsequent challenge injection of 100 mg of the same antigen intraperitoneally, or 60 mg intracardially, produced no symptoms of anaphylaxis.

While histamine shock can be produced in the guinea pig, rabbit, dog, and cat with symptoms very similar to those observed during anaphylaxis in these animals, in our experiments there was no evidence to indicate that histamine had any significant effect on any particular organ of the hamster. Death resulting from intracardial administration of 1.10 mg of histamine phosphate may have been due to the toxic effect of the drug on the vascular system of the animal. Previous workers (Wyman et al., 1956) have reported an LD₅₀ of 100 mg of histamine phosphate for the hamster using the intraperitoneal route of inoculation.

Variations observed in toxic doses of serotonin, leukocyte counts, complement titers and blood coagulation times in the hamster under ether anesthesia, cannot be explained. Likewise, it is not certain if similar variations would also occur in the absence of anesthesia.

SUMMARY

Using various routes of inoculation, the administration of up to 120 mg of ovalbumin to the hamster failed to sensitize the animal for anaphylaxis.

The administration of 0.495 mg of histamine phosphate was sufficient to produce nonfatal shock in the animal. Autopsy showed no anatomical changes.

Considerable variation in lethal doses of histamine phosphate and serotonin was observed in hamsters under anesthesia. Likewise, wide variations were observed in the leukocyte counts, complement titers, and blood coagulation times. These values remained essentially unchanged following administration of histamine phosphate and/or serotonin.

LITERATURE CITED

- Biggs, R. P. and R. G. Macfarlane. 1957. *Human Blood Coagulation and its Disorders*. 2nd ed., Oxford.
- Campbell, D. H., J. S. Garvey, N. E. Cremer and D. H. Susdorf. 1964. *Methods in Immunology*. W. A. Benjamin, Inc., New York.
- Good, R. A. and B. Campbell. 1945. Potentiating effect of anaphylactic and histamine shock upon herpes simplex virus infection in rabbits. *Proc. Soc. Exp. Biol. Med.* 59:305-306.
- Good, R. A. and B. Campbell. 1948. The precipitation of latent herpes simplex encephalitis by anaphylactic shock. *Proc. Soc. Exp. Biol. Med.* 68:82-87.
- Seegal, B. S. 1935. *Agents of Disease and Host Resistance*, F. P. Gay, ed., Charles C. Thomas, Springfield, Ill.
- Sherwood, N. P. 1951. *Immunology*, C. V. Mosby Co., St. Louis, Mo.
- Wyman, L. C., L. L. Drayseau and G. P. Fulton. 1956. Toxicity of histamine and dial with urethane in intact and adrenalectomized hamsters. *Proc. Soc. Exp. Biol. Med.* 92:467-468.