Anaphylactic Reaction in the Cat Following Intraventricular and Intravenous Injections of Antigen

We reported earlier that the administration of cellular and soluble antigenic materials into the cerebral cavity represents an effective route of stimulating the production of antibody[1,2]. The present study was primarily concerned with anaphylactic response in cats following injections of bovine γ-globulin (BGG) into the lateral ventricle of the brain, but anaphylactic reaction after i.v. administration of antigen was also investigated.

A permanent cannula[3] was implanted into the right lateral ventricle of the brain of 5 adult cats. 12 days after the operation, the animals were immunized with BGG (Armour). The first series of injections (15 mg of BGG per injection) was applied through the cannula directly into the cerebral cavity, with an interval of 26–31 days between the injections. The sensitization was then continued with i.v. injections (40 mg of BGG per injection). Since in this preliminary work there were no fatal casualities due to the immunization, the histological examination of various organs was not performed. The cats were sacrificed 3 weeks after the last i.v. injection. Serum was separated from the clotted blood, and cerebrospinal fluid obtained from cisterna magna. Brain and several parts of spinal cord were processed for histology, and stained with hematoxylin-eosin. Anti-BGG antibody was detected by quantitative precipitin reaction[4], passive hemagglutination using formalinized and tanned sheep red blood cells, and micro-immunoelectrophoresis[5]. The presence of sensitizing antibodies was demonstrated by passive cutaneous anaphylaxis[6]. For this purpose, saline, uniluted serum and cerebrospinal fluid, and serum diluted 1:2 and 1:4, were injected intradermally into the shaved dorsal skin of albino rats weighing 225 ± 25 g. The animals were challenged 4 and 16 h later with an i.v. injection of 1 mg of BGG dissolved in 1 ml of 0.5% Evans blue in saline. 60 min later the reactions were read on the inner side of the skin.

The results show that anaphylactic shock can be elicited in cats when BGG is used as antigen (Table). The mild shock was characterized by restlessness, scratching of the head, dyspnoea, tachycardia and urination. These symptoms occurred in cats No. 1 and 2 after the last i.v. injection of BGG. In severe shock, exophtalmos, dilatation of pupils, convulsions, salivation, vomiting, defecation, cyanosis of mucous membranes and prostration completed the list of anaphylactic symptoms. No fatal shock was observed in this experiment. Clinical signs disappeared gradually in the course of 70–90 min, but a state of depression and exhaustion persisted for several hours. The symptomatology of the anaphylactic reaction described here is consistent with that reported by McCusker and Aitken[7]. It should be emphasized that no signs of anaphylactic response were noted in cats following injections of BGG into the cerebral cavity. These negative results are probably referable to the slow absorption of antigen from the cerebral cavity[8]. Control normal cats given a single intraventricular or i.v. injection of antigen, and cats sensitive to BGG but challenged with unrelated antigen (bovine serum albumin) did not exhibit anaphylactic reaction.

The amount of precipitating antibody in the sera of sensitized cats was too small at the end of experiment to be detected by quantitative precipitin reaction and passive hemagglutination. However, immunoelectrophoretic analysis revealed one precipitin line in the region of immunoglobulins, and passive cutaneous reactions of moderate intensity (5–12 mm in diameter) were elicited in rats. All attempts to detect anti-BGG activity in cerebrospinal fluid or to provoke passive cutaneous reactions with it utterly failed. The histological examination of brain and spinal cord did not reveal inflammatory lesions, hemorrhages or other pathological changes.

Despite an abundance of data concerning the anaphylactic hypersensitivity, and the quantity and quality of antibody required to elicit anaphylactic response in various species[9], very little is known about the immune capacity of the cat. It has been claimed that the cat is a poor producer of antibody[10], and peculiarly resistant to sensitization[11]. The present report, however, clearly

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Severity of anaphylactic reaction in cats following intraventricular and i.v. injections of BGG

Cat No.	Intraventricular injections			Intravenous injections				
	No. of injections	Total amount (mg) of BGG injected	Anaphylactic reaction	No. of injections	Total amount (mg) of BGG injected	Anaphylactic reaction	No. of precipitin lines[a]	Mortality rate
1	7	105	None	2	80	Mild	1	0
2	6	90	None	3	120	Mild	1	0
3	6	90	None	3	120	Severe	1	0
4	6	90	None	4	160	Severe	1	0
5	6	90	None	5	200	None	1	0

[a] Revealed by immunoelectrophoresis.

indicates that this species is susceptible to anaphylaxis, although it seems at present that this sensitivity is inferior to that observed in other laboratory animals. Additional studies will be needed for a more precise evaluation of the biological properties of cat anaphylactic antibodies.

BESREDKA[12] was the first to introduce an immuno-neurological model by using the intracerebral route in studying the nervous origin of anaphylactic symptoms. His approach to the investigation of immune apparatus and its manifestations still represents the most provocative subject of experimental medicine, and undoubtedly deserves to be explored by means of all the technical and philosophical potentials of contemporary immunology and neurosciences[13].

Résumé. La γ-globuline bovine, en injections intraveineuses et dans le ventricule latéral du cerveau, a produit, chez le chat, l'apparition d'anticorps spécifiques dans la circulation et la sensibilisation déterminant une réaction anaphylactique d'intensité variable.

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Effect of Bilirubin on Glucose Oxidation in Red Cells

There has been ample evidence that the erythrocytes bind unconjugated bilirubin[1,2]. Bilirubin combined with human erythrocytes may be a factor in the shortened red cell survivals observed in erythrocytes pre-incubated in the solutions of unconjugated bilirubin[3]. The purpose of the present study is to elucidate the effect of bilirubin on glucose oxidation in erythrocytes in view of the fact that glycolysis is the main source of the red cell's energy.

The blood used in the study was obtained from healthy adult volunteers. Venous blood was obtained from the subjects with use of heparin as an anticoagulant. The blood was centrifuged at 1500 rpm for 10 min and the plasma and buffy coat were removed by aspiration. The packed erythrocytes were not subjected to further washing by buffer solution to avoid lowering ATP levels[4].

Experiment 1. One part of the packed erythrocytes was mixed with 1 part of either one of the following solutions: Solution A containing bilirubin; bilirubin (Daiichi Chemical Co., Tokyo) was dissolved in 0.1 *N* Na_2CO_3 solution at a concentration of 1.0%[5]. The bilirubin solution was then mixed with Krebs-Ringer phosphate buffer containing 1% human albumin (Sigma Chemical Company) to give a final concentration of bilirubin of 0.03%. The solution was freshly prepared and the pH was adjusted to 7.4 with 1 *N* HCl.

Solution B: Prepared as described above except that bilirubin was omitted. Each of the red cell suspensions thus prepared was divided into 2 equal portions. The first portion was incubated in the dark with occasional gentle agitations at 37 °C for 50 min, aiming at facilitating the binding of bilirubin with the red cells[1,2]. After the incubation, the red cells were resuspended without washing in Krebs-Ringer phosphate buffer (pH 7.4) to give a hematocrit reading of 0.40–0.50. The second portion of the red cell suspension was not incubated as described above.

$^8/_{10}$ ml of 1 of the red cell suspensions and 0.1 ml of ^{14}C-U-glucose[6] (125,000 dpm) containing 16 mg glucose were placed in a 25 ml flask equipped with a center well. A rubber stopper was attached to the flask. 1 ml of $^{14}CO_2$ trapping solution (1 part ethanolamine and 2 parts ethylene glycol)[7] was injected through the rubber stopper of the flask into the center well. These aliquots were incubated on a metabolic shaker and shaken at 45–50

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Effect of bilirubin and methylene blue on the glycolytic activities of the erythrocytes

	Experiment 1					Experiment 2				
	Erythrocytes		Erythrocytes; Bilirubin		*t*-test	Erythrocytes; Methylene blue		Erythrocytes; Methylene blue; Bilirubin		*t*-test
	$n = 6$ Mean	S.D.	$n = 6$ Mean	S.D.		$n = 6$ Mean	S.D.	$n = 6$ Mean	S.D.	
No preincubation	0.169	0.039	0.154	0.020	$p > 0.05$	3.37	0.43	2.25	0.14	$p < 0.01$
With preincubation	0.141	0.012	0.145	0.025	$p > 0.05$	3.82	0.50	2.25	0.25	$p < 0.01$

The glycolytic activities of the erythrocytes are expressed as percent of the initial radioactivities of the ^{14}C-U-glucose released as $^{14}CO_2$.