Lack of Specific Inhibition of Graft-versus-Host Reaction by Anti-Fab Serum

Anti-immunoglobulin sera (aIgS) have been shown to produce some effects on the immunological reactivity of lymphocytes. Anti-allotype sera transform lymphocytes[1]; high titre aIgS inhibits the adoptive secondary response[2]. In the action of these sera, the possibility of a reaction with a receptor arises. Therefore it is worth inquiring if they would act on a primary response, for instance on the graft-versus-host reaction (GVHR); an experiment of this nature may also elucidate the role of immunoglobulins in cellular immunity.

Methods. Sera. The following sera were employed: rabbit anti-mouse Fab serum (RaMFabS) containing 2.7 mg/ml precipitin, heated 10 min at 56 °C; rabbit anti-mouse Ig serum (RaMIgS) containing 1.2 mg/ml precipitin, heated 10 min at 56 °C; and normal rabbit serum (NRS), as control, heated 10 min at 56 °C. The RaMFabS was prepared against Fab fragments prepared by papain digestion of normal mouse IgG (NMIgG), Sephadex G75 filtration and DEAE cellulose chromatography, and contained 10% anti-Fc activity. The NMIgG was prepared by precipitation of normal mouse serum with 35% saturated ammonium sulphate.

Graft-versus-host reaction assay. CBA mouse lymph node or spleen cells were incubated in medium 199 with various concentrations of RaMFabS and 10% calf serum at 37 °C for 30 min; they were then washed and in some cases reincubated for 30 min with fresh rabbit serum absorbed with mouse red cells as a source of complement (RC′); they were then again washed and injected in various numbers into new-born F_1 (CBA × Balb/c) mice. The spleens of the recipients were weighed 10 days later.

Cytoxicity test. The technique of SANDERSON[3] modified by RUSZKIEWICZ[4], based on release of ^{51}Cr from CBA lymph node cells in presence of RC′, was used.

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Effect of anti-Fab serum on GVHR of adult CBA mouse spleen or lymph node cells in new-born F_1 (CBA × Balb/c) mice

Experiment No.	Incubation				No. cells injected per g	GVHR of cells incubated with		
	Cells Origin	Concentration per ml	RaMFabS Concentration (%)	RC′ Concentration (%)		RaMFabS	Control NRS	199 + RC′ alone
2	Spleen	5×10^7	2.5	0	5×10^6	+	+	+
3		6×10^7	2	33	2×10^7	+	+	+
		6×10^7	2	33	5×10^6	+	+	+
6	Lymph nodes	2.5×10^6	12.5	5.5	5×10^6	0		+
		2.5×10^6	12.5	5.5	5×10^5	0		0
8	Lymph nodes	2.5×10^6	12.5	5.5	5×10^6	0	0	+
		2.5×10^6	12.5	5.5	1.7×10^6			0

The sign + means a positive GVHR with a spleen index > 1.30[5].

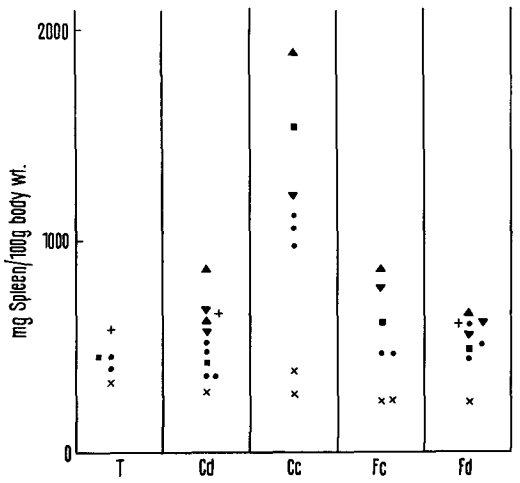

Fig. 1. Inhibition by anti-Fab serum of GVHR of lymph node cells. Experiment No. 6. T, control new-born (nb) spleen weight (mg/100 g body wt.). Cd, nb injected with 5×10^6 cells/g incubated with 199 alone. Cc, idem, but 5×10^7 cells/g. Fd, nb injected with 5×10^6 cells/g incubated with anti-Fab serum. Fc, idem, but 5×10^7 cells/g (inhibition). Each symbol corresponds to a different litter.

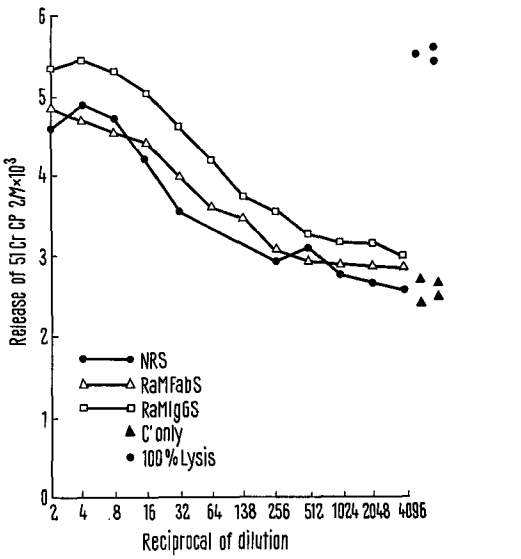

Fig. 2. Cytoxicity of rabbit anti-Fab, anti-Ig and normal sera on mouse lymph node cells in presence of 5.5% complement. At $^1/_8$ dilution, lysis > 50%.

Results and discussion. The Table shows that a GVHR was observed except where adequate concentrations of RaMFabS or NRS and RC′ were used. The GVHR of 5×10^6 cells/g body wt. injected was inhibited after incubation either with 12.5% RaMFabS and 5.5% RC′ or with 12.5% NRS and 5.5% RC′ (Figure 1) but not with medium 199 alone. Thus the mechanism of inhibition does not seem to depend on a specific reaction, but on the cytotoxicity of rabbit sera. Indeed, the cytotoxicity of RaMFabS, RaMIgGS, and NRS too, could be demonstrated at a concentration of 12.5% as shown in Figure 2.

Résumé. En recherchant une action des anticorps anti-Fab sur la réaction primaire des cellules immunologiquement compétentes, on a mis en évidence une inhibition de la réaction du greffon contre l'hôte par du sérum de Lapin anti-Fab de Souris: cette inhibition n'est cependant pas liée aux anticorps anti-Fab, mais à la cytotoxicité des sérums de Lapin aussi bien anti-Fab que normal.

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[7] Acknowledgements. I am grateful to Dr. N. A. Mitchison and to Dr. M. Ruszkiewicz for their helpful advice.

[8] Scholar of the Medical Research Council.

Effect of Hypothalamic Crude Extract on the Utilization of D-[^{14}C]-Glucose by the Anterior Pituitary, in vitro

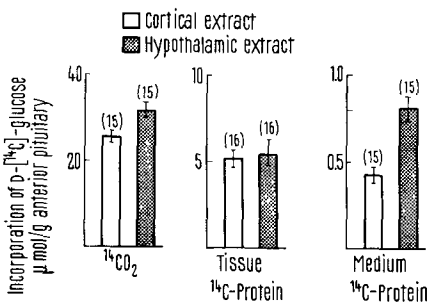
It is a well established fact that the hypothalamic control of the anterior pituitary gland is done by means of chemical substances known as releasing or inhibiting factors[1–3]. These substances are released into the hypophyseal portal vessels and act directly on the adenohypophysis to increase or decrease the release of each trophic hormone.

Evidence afforded by in vivo and in vitro experiments indicates that the hypothalamic extract (HE), in addition to controling the release of the anterior pituitary (AP) hormones, may also influence their synthesis[4–7].

Experiments were undertaken in this laboratory to find out whether the action of the hypothalamic releasing factors is accompanied by changes in the metabolism of energy delivering substracts. The present paper will report the effects of crude HE in the utilization of glucose by AP incubated in vitro, in conditions similar to those in which the HE are already known to effect hormone release[8,9].

Albino male rats weighing 200–250 g were killed by decapitation. Anterior pituitaries were quickly removed, separated from the posterior lobe, weighed, hemisected and placed in the main compartment of incubation flasks provided with a center well for CO_2 collection and with a self-sealing rubber stopper. The incubation medium consisted of Krebs-Henseleit bicarbonate buffer, to which either HE or cerebral cortex (CE) was added. In order to have a paired type experiment, one-half of each pituitary was placed in the experimental flask and the other in the control flask. For preparation of the extracts, hypothalami or cerebral cortex were homogenized in cold 0.1 *N* HCl and centrifuged at 2500 rpm for 30 min at room temperature just before each experiment. The supernatant was separated and the pH adjusted to 7.4 by adding dropwise 1.0 *N* NaOH.

Details of the incubation procedure are indicated in the Figure. At the end of the incubation period 0.3 ml of 30% KOH was injected through the rubber stopper into the center well, and, in a similar manner, 0.3 ml of 3 *M* H_2SO_4 was added to the incubation medium in the main compartment. After allowing at least 30 min for absorption of the $^{14}CO_2$, the contents of the center well were quantitatively transferred to a volumetric flask. An aliquot of this solution was precipitated as $Ba^{14}CO_3$ and its radioactivity measured in a gas flow counter (Model D47, Nuclear Chicago Co.). Corrections for self-absorption were made according to Karnowsky et al.[10]. After washing the AP several times with distilled water, the glands were homogenized in cold 10% trichloroacetic acid. The precipitated ^{14}C-protein was then transferred



Effect of hypothalamic extract on the utilization of D-[^{14}C]-glucose by anterior pituitary gland. The incubation medium consisted of 2 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 12 µmol of D-[^{14}C]-glucose (0.8 µc/flask) plus 0.2 ml of either hypothalamic or cerebral cortex extract. Anterior pituitaries (4 halves per flask) were incubated for 3 h at 37 °C with constant shaking and with 95% O_2 – 5% CO_2 as the gas phase. Values (mean ± S.E.M.) are expressed as µmoles of glucose incorporated per g of wet tissue per 3 h incubation. The number of experiments is indicated in parentheses.

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