mammalian blood have proved to cause immunosuppression in various in vivo and in vitro tests, but Nip has been shown to be different, demonstrating no crossreactivity with the following; $\alpha$ fetoprotein, as determined by radioimmunoassay, pregnancy associated $\alpha_2$ macroglobulins, $\beta_1$ glycoprotein, C reactive protein, as determined by immunoelectrophoresis. The same was true of fibrinogen degradation products and several others that were extracted from liver tissue, lymphatic tissue, macrophages, thymus and tumors, as determined by absorption of anti-Nip antiserum and the quantitative examination of this serum against Nip in the Elisa method. In many respects Nip is similar to immunoregulatory $\alpha$ globulin (IRA)[14] but differs from it in its strong immunosuppressive activity on B cells (immunological cross reactivity, however, has not been tested).

At present it is not known where and how Nip is produced nor do we know what causes elevation of Nip levels. In spite of these uncertainties it is clear that Nip and/or similar serum factors praticipate in the control mechanisms of the immune response.

1 The author wishes to thank Prof. G. Klein, Karolinska Institute, for his help and suggestions. - Supported by a grant from the Lil and Ben Stein Organ Transplantation Foundation.
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## The effect of continuously cooling the hypothalamic preoptic area on antibody titre in the rat

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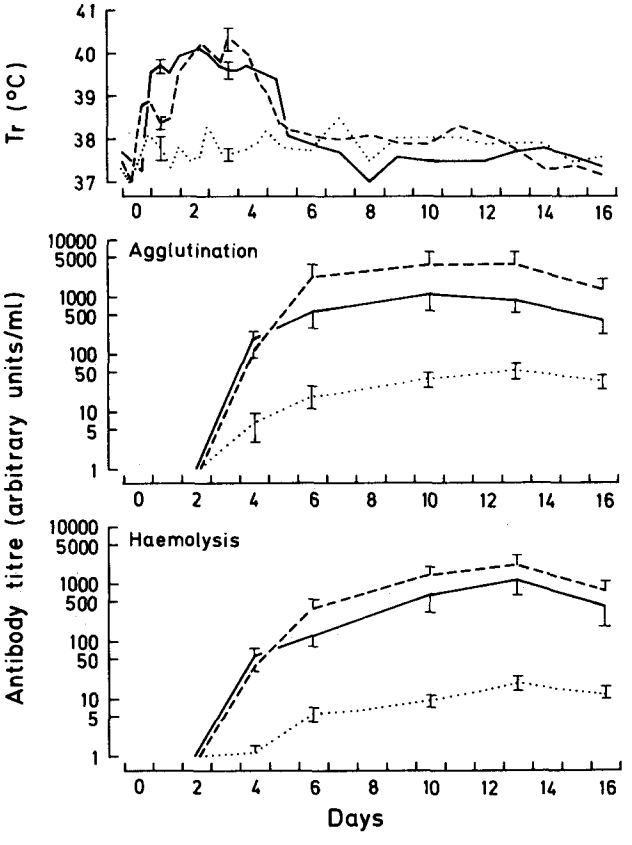
*15 October 1981*

*Summary.* Continuous cooling of the hypothalamic preoptic area for 5 days, as well as sublethal infection with *Salmonella enteritidis*, increased the titre of antibodies against sheep erythrocytes, suggesting that the febrile response stimulates the humoral immune response.

It has long been thought that fever may stimulate the immune system[2], though passive hyperthermia often inhibits it[3,4]. Some support for this view was recently obtained in experiments in which intermittent fever, either induced by an endogenous pyrogen[5] or simulated by cooling the hypothalamic preoptic area[6], increased the titre of antibodies. Many infectious diseases elicit, however, continuous fever, and this sustained increase in body temperature might harm the immune system. In the present work, therefore, we studied the effect of continuous fever, simulated by cooling the hypothalamic preoptic area, and we show that it also stimulates the humoral immune response.

*Methods.* During the spring, white male rats weighing about 350 g were maintained at an ambient temperature of 23 °C with natural illumination. Food and water were continuously available. In some of the rats, a thermode was chronically implanted into the preoptic area, and the animals were then fixed to an antirotatory device[7]. 1 week afterwards, these animals were randomly divided into 2 groups, hypothalamus-cooled and control, of 7 animals each. The rest of the animals, all without thermodes, were




Body temperature and titre of antibodies against sheep erythrocytes in 6 hypothalamus-cooled (solid line), 5 infected (broken line) and 13 control animals (dotted line). At noon on day 0, all animals were immunized with sheep erythrocytes, while those of the infected group were additionally injected with live *S. enteritidis* (0.0002 mg bacterial dry weight). The thermodes of the animals of the hypothalamus-cooled group were perfused with cold water from day 0 at 18.00 h until day 5 at 10.00 h. The vertical lines are SE's.

divided in the same manner into 3 groups of 6 animals each: infected, heat-exposed and control. All animals were then injected i.p. with 1 ml of a 10% suspension of washed sheep erythrocytes, while those of the infected group were additionally injected with a sublethal dose of live *Salmonella enteritidis* (0.0002 mg bacterial dry weight)[8]. 6 h later, the animals of the heat-exposed group were placed at an ambient temperature of 37 °C, while the thermodes of the hypothalamus-cooled group were perfused with water the temperature of which was regularly adjusted to induce a change in body temperature similar to that elicited by *S. enteritidis*[8]. Both experimental treatments, heat exposure and cooling of the hypothalamus, were discontinued after 5 days, and the animals were followed for another 11 days. Throughout the experiments, body temperature was measured every 8 h, and blood samples were taken at various time intervals. All methods, including the determination of the titre of antibodies against sheep erythrocytes, have been described in detail elsewhere[6].

As there were no significant differences between the control animals with and without thermodes in any of the parameters studied in this work (see also Banet et al.[6]), these animals were pooled into a single control group. Furthermore, because 4 of the heat-exposed animals died during their exposure, though their rectal temperature never increased above 40 °C, the results of this group are not included in this report. 2 other animals, one each from the infected and hypothalamus-cooled groups, also died and their results were excluded. The cause of their death is not known, but it was noted that in both animals body temperature increased to nearly 41 °C on the 3rd experimental day.

*Results.* The figure summarizes the results. During the 1st 5 experimental days, the animals of the hypothalamus-cooled and infected groups maintained higher core temperatures (mean ± SE: 39.7 ± 0.1 and 39.5 ± 0.2 °C, respectively) than those of the control group (37.8 ± 0.2 °C; $p < 0.001$, t-test). The animals of the hypothalamus-cooled group tended to have a lower titre of antibodies than those of the infected group but the highest titres achieved were not significantly different. The highest titres of both groups of experimental animals were, however, significantly higher than those of the control ones ($p < 0.005$).

*Discussion.* Intermittent cooling of the hypothalamus stimulates the humoral immune response[6]. Since antibody titre increased after continuous and prolonged cooling of the hypothalamus, the present results show that the production of antibodies is not impaired when a high body temperature is sustained. Endogenous pyrogen has also been shown to increase the titre of antibodies[5], and this effect appears to be similar to that induced by intermittent cooling of the hypothalamus[6]. Since cooling the hypothalamus simulates the febrile response[7], it is likely that the main effect of pyrogen on antibody titre is due to its febrile rather than to any other of its actions.

Infection with *S. enteritidis* also increased the titre of antibodies. The responses of a host to a pathogen are, of course, too complex to allow a simplistic interpretation of this increase in antibody titre. However, since the effect of infection on antibody titre was similar to that of cooling the hypothalamus, it is possible that a large part of the former effect may be attributable to the febrile action of the endogenous pyrogen induced by the pathogen. These experiments thus support the view that a sustained febrile response stimulates the humoral immune response, but present no evidence as to whether this effect is mediated by the increase in body temperature or by other nonthermal aspects of the febrile response[6,8].

1 We thank Dr W. Mannheim from the Institute of Hygiene for kindly supplying the cultures of *S. enteritidis*. This work was supported by the Schwerpunktprogramm Temperaturregulation und -adaptation of the Deutsche Forschungsgemeinschaft.
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## Histamine binding to $H_2$ receptors stimulates phospholipid methylation in mast cells

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*Summary.* Rat peritoneal mast cells incubated in vitro in the presence of L-[methyl-$^3$H] methionine and exposed to histamine undergo a rapid but transient increase in phospholipid methylation. By using specific $H_1$- and $H_2$-receptor antagonists, and histamine analogues differing in their $H_2$-receptor agonist potency, it has been demonstrated that this metabolic event is dependent on histamine binding to $H_2$-receptors.

There is substantial evidence that histamine release from basophils and mast cells is modulated by histamine itself through a $H_2$-mediated effect which is apparently coupled to the activation of adenylate cyclase[2,3]. The mechanism(s) responsible for this coupling remain to be determined. Hirata et al.[4] have recently reported that binding of the $\beta$-adrenergic receptor to its agonist, L-isoproterenol, stimulates phospholipid methylation and translocation which, in turn, increases membrane fluidity and coupling of the $\beta$-adrenergic receptor with adenylate cyclase in rat reticulocyte ghosts. Since in mast cells stimulation of both $H_2$ and $\beta$-adrenergic receptors results in adenylate cyclase activation[3], we have investigated whether binding of histamine to the $H_2$-receptors also increase phospholipid methylation in these cells.

*Material and methods.* Purification of mast cells. Male Sprague-Dawley rats (200-250 g) were anesthetized with ether, exsanguinated by cutting the carotid arteries and laparatomized; peritoneal cells were harvested in a medium (MCM buffer) containing 150 mM NaCl, 3.7 mM KCl, 3.0 mM $Na_2HPO_4$, 3.5 mM $KH_2PO_4$, 0.9 mM $CaCl_2$, 5.6 mM dextran, 0.1% (w/v) bovine serum albumin (BSA), 0.1% gelatin and 10 units $ml^{-1}$ heparin (pH 6.8). After purification by a BSA density gradient centrifugation method[5], mast cells of 90% or greater purity were obtained. Cell concentration was determined by a standard microhae-