by 21 days, when the secondary stimulus was administered, primary circulating titers (Figure 1, A) were within about 1 $\log_2$ titer of the primary plateau value noted for the young NZB mice. The secondary hemagglutinin response of these overtly autoimmune NZB mice was rapid (Figure 1), and the peak level achieved was comparable to that of the young NZB and BALB/c animals (Figure 2). At this time, the 7 surviving old mice were all strongly Coombs positive with hematocrits averaging 31% (range 17–37).

Background hemagglutinin titers. Anti-SRBC agglutinin titers of sera from unimmunized 35-day-old NZB and BALB/c mice and 12- to 14-month-old NZB mice are presented in the Table. Young NZB mice failed to show the presence of agglutinins at serum dilutions of 1:6, whereas half of the young BALB/c sera manifested agglutinin activity at this dilution. Most of the unimmunized old NZB animals tested showed SRBC titers of less than 1:6 although sera from 4/39 of these mice agglutinated SRBC at dilutions of 1:12 to 1:48. These differences observed in background responses did not appear to be related to the differences in responses obtained following active immunization.

Discussion. Primary hemagglutinin titers of young adult NZB mice, assayed both in the presence and absence of 2-mercaptoethanol, were significantly higher than those obtained with the sera of age- and sex-matched BALB/c mice. This finding was consistent with previously observed differences in spleen antibody plaque formation between the 2 mouse strains and tended to support the concept of an immunologic hyperresponsiveness of NZB mice to this antigenic stimulus[2]. Such differences in response diminished, however, following secondary and tertiary immunization, a possible consequence of similar capacities of these 2 mouse strains to respond to feed-back controls limiting further antibody formation[10]. The normal secondary hemagglutinin response of old NZB mice was also in agreement with previous results obtained by use of the JERNE plaque assay[3] and likewise indicated the possible presence of undiminished numbers of memory cells.

The present findings of delayed appearance of circulating agglutinins in old NZB mice after primary immunization is analogous to earlier observations of depressed numbers of plaque-forming cells[2]. The reason for this delayed response has yet to be elucidated. Carbon clearance rate studies[11] have indicated increased reticuloendothelial system (RES) activity in these animals. However, a causal relationship between this increased RES activity and immune depression has not been established, though defects in antigen processing may conceivably be present in these animals. An alternative explanation for the delayed humoral response of old NZB mice following primary immunization might be the diminished presence of antigen-sensitive cells[2]. It is known, for example, that antigenic information may persist for some time following immunization[10]. Thus, as additional antigen-sensitive cells became available these could be triggered to antibody production until normal circulating titers were reached. Impaired mitosis of initially stimulated progenitor cells could also contribute to delayed antibody formation. Further studies are required to clarify the mechanism of immunodepression manifest in the overtly autoimmune animal[12].

Zusammenfassung. Es wird gezeigt, dass der Gehalt an Serumantikörpern nach Immunisation mit Schaferythrozyten in jungen NZB-Mäusen im Vergleich zu BALB/c-Stämmen grösser ist. Diese Unterschiede wurden nach der zweiten und dritten Antigeninjektion geringer. Bei alten Coombs-positiven NZB-Mäusen wird eine Verzögerung der Reaktion nach der ersten, jedoch eine normale Reaktionszeit nach der nun folgenden Injektion gefunden. Die Ergebnisse entsprechen den Resultaten mit JERNE's[1] Technik bei Reaktionsuntersuchungen antikörperbildender Zellen.

SRBC agglutinin titers of sera from unimmunized NZB and BALB/c mice

Group	No. of mice tested	Titer				
		<1:6	1:6	1:12	1:24	1:48
Young NZB	23	23[a]	0	0	0	0
Young BALB/c	52	26	26	0	0	0
Old NZB	39	26	9	1	2	1

[a] Number of mouse sera showing designated titer.

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Further Evidence for the Existence of a Hypothalamic Follicle Stimulating Hormone Synthesizing Factor

Previous reports by CORBIN and STORY[1] and CORBIN and DANIELS[2] indicated that, in the rat, the hypothalamus may control the synthesis of pituitary follicle stimulating hormone (FSH), as well as its release. A single i.v. administration of stalk-median eminence (SME) extract caused depletion of pituitary FSH[2]. A second injection, made at the time of maximum pituitary FSH depletion (45 min), induced the resynthesis of hypophysial FSH at a rate faster than that which occurred after a single injection of the extract. The hypothesis that a hypothalamic FSH-synthesizing factor (FSH-SF) exists has been tested in studies employing male rats with depressed stores of pituitary FSH induced by lesions of the median eminence (ME).

The SME's were derived from 60-day-old, intact, normally cycling Sprague-Dawley (S-D) female rats. The tissue was homogenized in 0.1 *N* HCl, centrifuged, and the supernatant material was immersed in a boiling water bath for 10 min and then diluted with the acid to a concentration corresponding to 2 SME/ml. In a modification of the in vivo procedure of DAVID et al.[3] for the evaluation of FSH-releasing factor (FSH-RF), the material was injected into the jugular vein of intact male rats or male rats bearing ME lesions for 7–10 days (S-D, 200 ± 10 g: 5 rats/group). The lesions had been made with a direct current of 5 ma/15 sec. An earlier time study had shown that pituitary FSH became significantly depressed 7 days after the ME lesion, and remained so for 60 days.

The animals were decapitated 45 min after injection and their pituitaries were removed, pooled, weighed, homogenized and diluted with physiological saline for use in the FSH assay of STEELMAN and POHLEY[4]. 2 or 3 levels of the NIH-FSH-S4 reference standard were used; the total doses ranged from 25 µg to 100 µg, with a two-fold dose interval. The male pituitary homogenates were assayed at total doses of 1.5 mg and 3.0 mg wet weight. 5 rats were employed for each dose level. Estimates of pituitary FSH concentrations were calculated by the methods of BLISS[5] and the results of replicate assays were combined by the methods of BLISS[5] and SHEPS and MOORE[6]. The results are expressed in terms of NIH-FSH-Sl. 2 different studies were performed: a) the effect of 2 SME on pituitary FSH concentration of intact and ME lesioned rats and b) SME dose-response in intact and ME lesioned rats.

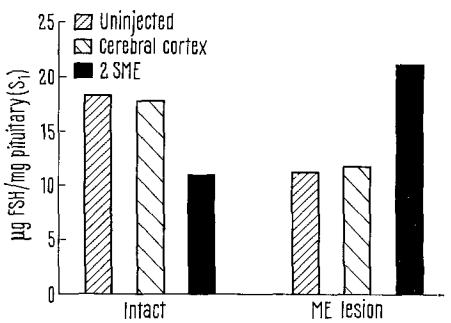

Fig. 1. Effect of 2 SME on pituitary FSH of intact and ME lesioned mature male rats.

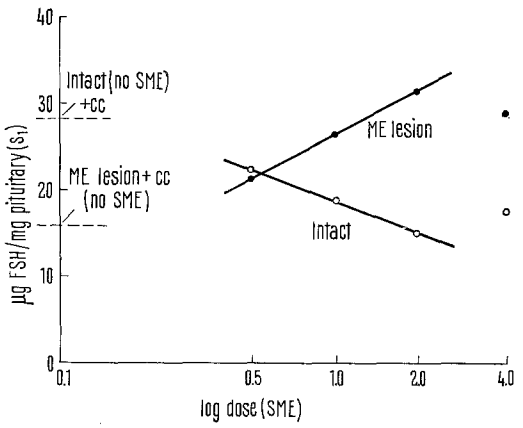

Fig. 2. Dose-response for rat SME: effect of various doses of SME on pituitary FSH of intact and ME lesioned mature male rats.

Figure 1 demonstrates the effect of 2 SME on pituitary FSH concentration of intact and ME lesioned rats. Each bar represents the geometric mean of 4 separate, but identical, replications. The i.v. administration of 2 SME to the intact rat resulted in a significant ($p < 0.01$ vs. intact + cerebral cortex) decrease in pituitary FSH concentration. In contrast, the ME lesioned rat, which has significantly ($p < 0.01$ vs. Intact, uninjected) reduced concentration of pituitary FSH, responded with a significant ($p < 0.01$ vs. ME lesion + cerebral cortex) increase in pituitary FSH concentration when injected with the same SME dose. Figure 2 demonstrates the effects of various doses of SME on pituitary FSH concentration in either the intact or ME lesioned rat. A log dose-response relationship exists for pituitary FSH depletion in the intact rat and for pituitary FSH repletion in the ME lesioned rat.

These data appear to support the contention that the hypothalamus can control both the release and synthesis of pituitary FSH. MARTINI (personal communication), employing the ME lesioned rat with depressed pituitary FSH stores, as described above, recently has corroborated the findings represented by Figure 1. Additional support for the concept of a hypothalamic FSH-SF recently was provided by EVANS and NIKITOVITCH-WINER[7] who demonstrated hypophysial and ovarian reactivation by ME extracts given to hypophysectomized rats bearing pituitary autografts under the kidney capsule.

It is not known whether the hypothetical FSH-SF is identical with the releasing factor for FSH, but the data suggest strongly that the hypothalamus does indeed possess a separate mechanism for regulating the synthesis of pituitary FSH. Furthermore, whether release or synthesis occurs in response to injection of SME appears to depend on the pre-treatment level of pituitary FSH. This relationship, evident in the present study, had been surmised in our previous double SME injection experiment[2]. Replicated dose-response studies, as well as time studies in the ME lesioned rat, are in progress and will be the subject of a future report.

Résumé. Après une periode de 45 min, l'injection d'extraits hypothalamiques dans la veine d'un rat mâle intacte provoque une chute maximale du niveau FSH hypophysaire; dans un rat mâle ayant une lésion du ME et un niveau de FSH hypophysaire inférieur à la normale, la même dose d'extrait hypothalamique fait hausser au maximum le niveau de FSH hypophysaire.

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