Par ce procédé, les résultats positifs de précipitation ont été observés après 20–25 min. La méthode est réellement plus rapide que les autres, mais elle ne peut rendre service qu'en cas d'analyses de protéines caracterisées par la migration électrophorétique vers l'anode, ce qui les mène à la rencontre des immunoglobulines spécifiques qui se déplacent à contre courant. Sous cette forme la méthode n'est pas utilisable pour les protéines cathodiques.

Compte tenu du problème, nous proposons une modification qui pourra être praticable en cas d'analyses accélérées des protéines cathodiques, séparement ou en mélange avec des protéines anodiques. Dans ce but nous avons utilisé le gel d'agar à 1,5% dans un tampon véronal sodique à pH 8,2[17, 18]. L'analyse est réalisée en deux temps:

1. Sur des plaques de gel solidifié, on découpe deux rangées de réservoirs, perpendiculairement au grand axe des plaques (réservoirs de 2 mm de diamètre et distance de 5 mm entre les rangées). Les réservoirs cathodiques sont remplis par les protéines recherchées; dans des réservoirs du côté anodique on introduit l'immunsérum précipitant, soumettant les plaques à l'électrophorèse à 20 V/cm, 2,5 mA/cm (Figure 1). La précipitation peut se faire au cours de l'électrophorèse pour les protéines concentrées, ou peu après pour les protéines diluées.

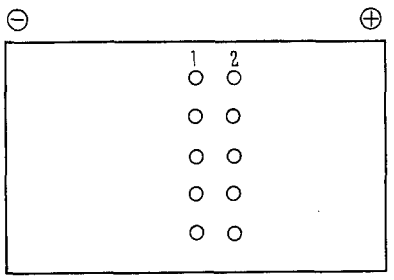

Fig. 1. Plaque avant électrophorèse. 1. Antigènes. 2. Immunsérum.

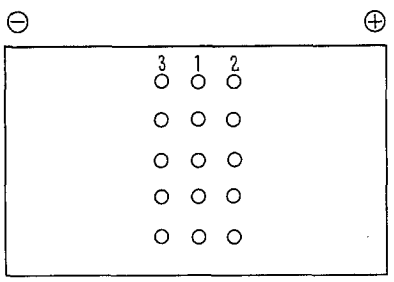

Fig. 2. Plaque après électrophorèse. 1. Antigènes. 2. Immunsérum. 3. Immunsérum.

2. Après 25 min, on coupe le courant électrique et on forme une troisième rangée de réservoirs (du côté cathodique qui contenait les antigènes étudiés) dans lesquels on introduit l'immunsérum précipitant (Figure 2). La précipitation se fait par double diffusion. Le temps d'apparition des résultats positifs est présenté au Tableau.

La modification proposée peut se révéler très utile en ce qui concerne l'analyse accélérée aussi bien des antigènes que des anticorps et notamment dans les cas suivants: a) Titrage des anticorps dans des échantillons du sang des animaux immunisés (vivants) avec la distinction simultanée du niveau des anticorps anti-protéines anodiques et anti-cathodiques. Dans une seule journée de travail cette modification permet de prélever des échantillons du sang, de titrer les anticorps et même de saigner les animaux. b) Titrage de protéines anodiques et cathodiques dans un mélange donné. c) Détermination de la pureté de préparations d'antigènes et d'immunoglobulines. d) Modification présentée en dehors du titrage, met en évidence l'homogénéité ou hétérogénéité des substances examinées, bien qu'elle soit moins utile dans l'étude de fractions protéiques, mais dans ce cas l'immunoélectrophorèse peut être d'un grand recours.

Summary. A new modification of agar immunoprecipitation is described. The modification allows one to shorten the time of precipitation of proteins which at pH 8.2 migrate towards anode or cathode. It can also be used for the titration of antibodies.

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16 mars 1970.

[1] E. GRASSET, Ann. Inst. Pasteur *91*, 162 (1956).
[2] A. CROWLE, J. Lab. clin. Med. *55*, 593 (1960).
[3] O. OUCHTERLONY, Acta path. microbiol. scand. *25*, 186 (1948).
[4] J. OUDIN, Ann. Inst. Pasteur *89*, 531 (1955).
[5] C. OAKLEY, J. Path. Bact. *65*, 49 (1953).
[6] S. ELEK, Br. J. exp. Path. *31*, 358 (1950).
[7] G. MANCINI, Immunochemistry *2*, 235 (1965).
[8] J. FEINBERG, Nature *177*, 530 (1956).
[9] M. CESKA, Immunology *15*, 837 (1968).
[10] PH. RÜMKE et J. C. BREEKVELDT-KIELICH, Vox sang. *16*, 486 (1969).
[11] V. GHETIE, Immunochemistry *4*, 467 (1967).
[12] E. V. CHERNOKHVOSTOVA, Biokhimia *33*, 1183 (1968).
[13] B. J. CULLIFORD, Nature *207*, 1092 (1964).
[14] J. FEINBERG, Int. Arch. Allergy *33*, 120 (1968).
[15] Z. MAREK, K. JAEGERMAN et B. TUROWSKA, Folia med. cracov. *1*, 83 (1964).
[16] O. PROKOP, D. SCHLESINGER et H. FALK, Dt. Gesundh Wes. *18*, 760 (1963).
[17] P. GRABAR, *Analyse immunoélectrophorètique* (Masson, Paris 1960).
[18] J. SCHEIDEGGER, Allergy appl. Immun. *7*, 103 (1955).

Circulating Hemagglutinins of New Zealand Black Strain Mice Immunized with Sheep Erythrocytes

Experiments employing the JERNE[1] plaque assay have demonstrated young adult New Zealand Black (NZB) strain mice to be hyperresponsive to primary immunization with sheep erythrocytes whereas old Coombs positive animals manifested marked immunodepression[2]. In the case of overtly autoimmune NZB mice, production of 7S and 19S antibody-forming cells during the secondary response was unimpaired[3]. The present report is an attempt to determine analogous aspects between these observed antibody-forming cell patterns and features of the circulating antibody response following a similar immunization regimen. This appeared of added interest

in view of conflicting data from a number of laboratories with regards to circulating antibody differences among these animals[4–6].

Twenty-six 5-week-old NZB and 26 BALB/cJ mice and ten 12- to 14-month-old Coombs positive NZB mice were immunized by i.p. injection of 1.5×10^9 saline-washed sheep erythrocytes (SRBC) and individual sera assayed periodically for total and mercaptoethanol (ME)-resistant agglutinins by a previously described microtiter assay[7]. Statistical evaluation of titer values was carried out by the Student *t*-test. All experimental animals were tested periodically for direct Coombs reactions[8].

Results. Primary responses of young adult NZB and BALBO/c mice. 4 days after primary immunization (Figure 1, A and B), circulating antibody titers of young adult NZB and BALB/c mice were essentially identical, and most of the antibody was ME-sensitive. By 7 days, however, total and ME-resistant antibody levels of sera from young NZB mice evidenced an increase over BALB/c values, which persisted over the 46-day-period of the primary response. Evaluation of these data indicated that the differences between NZB and BALB/c primary titers were statistically significant ($p < 0.01$–0.001). After day 17 of the primary response, ME-treated NZB mouse sera (Figure 1, B) showed antibody activity 1–2 titers greater than untreated sera (Figure 1, A), an observation for which we at present have no clear explanation. This mercaptoethanol-associated increase was not observed with ME-treated primary sera of either young BALB/c or old NZB mice.

Secondary and tertiary responses of young adult NZB and BALB/c mice. Following a second SRBC injection 47 days post-primary immunization, young NZB and BALB/c mice evinced similar titer increases (Figure 2) although the absolute differences in peak levels (day 56, Figure 2) remained significant by the *t*-test ($p < 0.01$). By 3 weeks following secondary stimulation circulating titers were similar for both mouse strains. Average titers attained were virtually identical following a third SRBC injection administered on day 138 (Figure 2). At this time the NZB mice showed no evidence of an autoimmune hemolytic process, presenting negative Coombs tests and normal hematocrit values (average 49%, range 48–51). Parenthetically, the mercaptoethanol enhancing effect, noted in the case of NZB primary sera (Figure 1), was observed to occur only infrequently following secondary administration of SRBC and not at all following tertiary immunization.

Primary and secondary responses of old NZB mice. Old NZB mice manifested a delayed total (Figure 1, A) and ME-resistant (Figure 1, B) primary antibody response, but the usual sequence pattern of appearance of ME-sensitive and of ME-resistant antibodies[9]. Unlike the responses observed with the young mice, no antibody was present at 3 days post-immunization, and at 7 days all antibody remained mercaptoethanol-sensitive. However,

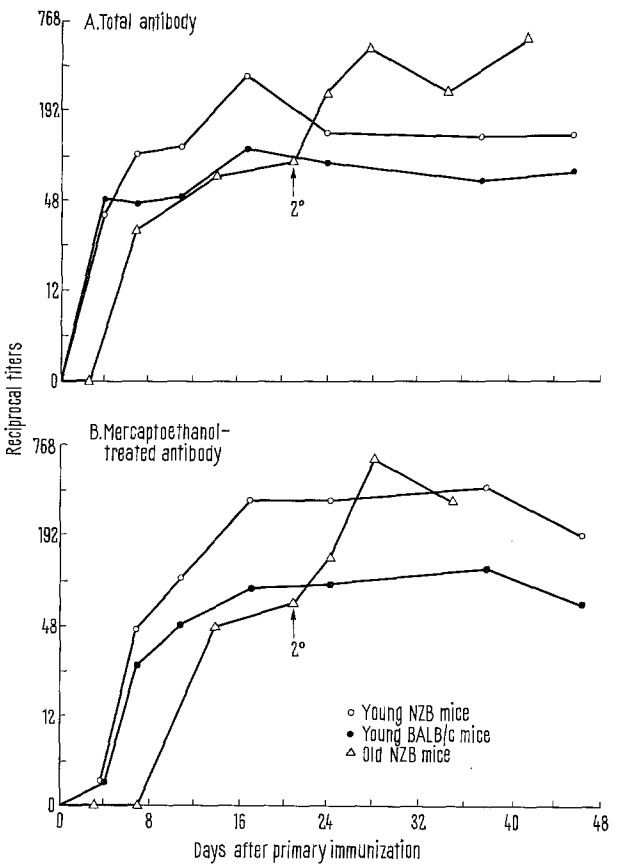

Fig. 1, A. Total reciprocal hemagglutinin titers for young NZB (○), young BALB/c (●), and overtly autoimmune, old NZB (△) mice, following i.p. injection of 1.5×10^9 sheep erythrocytes. 2° → indicates time of administration of a second SRBC dose to mice in the old NZB group. B. Similar parameters as in A except responses represent titers after addition of 0.05 *M* 2-mercaptoethanol to serum in the first dilution well.

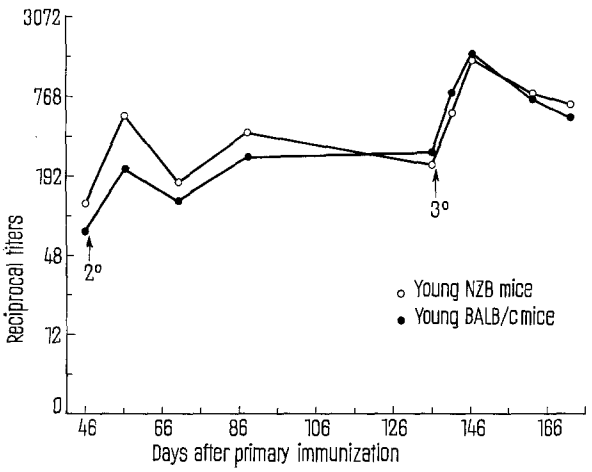

Fig. 2. Secondary (2° →) and tertiary (3° →) total hemagglutinin titer responses of young NZB and BALB/c mice. Points represent average values for the same mice presented in Figure 1.

[1] N. K. Jerne, A. A. Nordin and C. Henry, in *Cell-Bound Antibodies* (Ed. B. Amos and H. Koprowski; Wistar Institute Press, Philadelphia 1963), p. 109.
[2] J. I. Morton and B. V. Siegel, J. Reticuloendothel. Soc. *6*, 78 (1969).
[3] J. I. Morton and B. V. Siegel, Immunology *16*, 481 (1969).
[4] J. Baum, Clin. exp. Immun. *5*, 251 (1969).
[5] J. C. Cerottini, P. H. Lambert and F. J. Dixon, J. exp. Med. *130*, 1093 (1969).
[6] J. C. Salomon and J. Benveniste, Clin. exp. Immun. *4*, 213 (1969).
[7] B. V. Siegel and J. I. Morton, Proc. Soc. exp. Biol. Med. *123*, 467 (1966).
[8] L. C. Norins and M. C. Holmes, J. Immun. *93*, 897 (1964).
[9] F. L. Adler, J. Immun. *95*, 26 (1965).

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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[10] J. W. UHR and M. S. FINKELSTEIN, Progr. Allergy *10*, 37 (1967).
[11] J. I. MORTON and B. V. SIEGEL, Proc. Soc. exp. Biol. Med. *133*, 1055 (1970).
[12] Supported by USAEC Contract No. RLO-1927-47.

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).