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Preliminary Report

Anti-salmonella Substance of Rabbit Macrophages*

Studies on the antibacterial substances of guinea pig leucocytes have been carried out in this laboratory for about 10 years (Skarnes and Watson, 1957; Amano, 1961, 1962). Very recently studies have been extended to rabbit leucocytes as well as to macrophages. The anti-salmonella substance was found in rabbit macrophages and it increases after the injection of living cells of avirulent *Salmonella choleraesuis*.

Macrophages were collected from rabbit lungs according to the method of Oshima, Myrvik and Leake (1961). In this method a first injection of heat killed BCG in Freund adjuvant, subcutaneously, was given and a month later a second injection of heat killed BCG in saline, intravenously. In the interval between the two injections, rabbits were immunized with either live or dead vaccine and conrtol rabbits were not immunized. For immunization with live vaccine, 5×10^5 cells of *Salm. choleraesuis* (strain 1348) were administered subcutaneously on the 20th day after the first BCG injection and 2×10^6 cells of *Salm. choleraesuis var.* Kunzendorf (strain 5210) intravenously on the 24th day. For immunization with dead vaccine, four immunizations (2×10^8, 2×10^8, 5×10^8, 1×10^9 cells of the formalinized strain 5210) were made every 6 days during the interval. Strain 1348 of *Salm. choleraesuis* was very susceptible to the bactericidal action of normal fresh rabbit sera while strain 5210 was not susceptible to it.

After collection, macrophages were first extracted with M/15 phosphate buffer at pH 7.0 (10^8 cells per ml) by repeated freezing and thawing and the total volume was doubled with saline before centrifugation. The washed residue was extracted twice with saline containing M/4 acetic acid also by repeated freezing and thawing (the pH of the extract was 3.2) and then the washed residue was further extracted with 0.2 N hydrochloric acid (the pH of this extract was 0.8). The extracts were each neutralized (the total volume was doubled with M/15 phosphate buffer, pH 7.0) and the supernatants after centrifugation were each assayed for antibacterial activity.

The neutral extract of the native macrophages (N-N) was active against *E. coli B*, *Vibrio tyrogenus*. and strain 1535 (specific rough)of *Salm. choleraesuis*. The acetic acid extract (N-A) was also active against these three organisms and also the Vollum (virulent) strain of *B. anthracis*. Strains 1348 and 5210 of *Salm. choleraesuis* were not susceptible to N-N and N-A.

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The activities of the hydrochloric acid extracts (N-H) were assayed by measuring their viable counts and their turbidities in a Coleman junior spectrophotometer. The cells of strains 1348 and 5210 were each suspended in 1:10 dilutions of nutrient broth (pH 7.5) and 1.0 ml of N-H was added to 5.5 ml of the bacterial suspension. The optical density of the final mixture was about 0.3 at 550 mμ.

As shown in Figs. 1 and 2, N-H of macrophages from immunized rabbits with live vaccine (N-H-L) was strongly bactericidal as well as bacteriolytic. The activities of the N-H of macrophages from normal rabbits (N-H-N) or from animals immunized with dead vaccine (N-H-D) were not as strong especially when measured by the viable count method. There was no agglutination of bacterial cells and the bacteriolytic effect of N-H-L was reconfirmed by microscopic counting with a counting chamber. Eighty seven per cent reduction of the total bacterial count was observed. In addition, the lysozyme/EDTA spheroplasts of strain 5210 in a sucrose medium were lyzed by the addition of a minute amount of N-H-L. The result indicates that N-H-L damages the cytoplasmic membrane of bacteria.

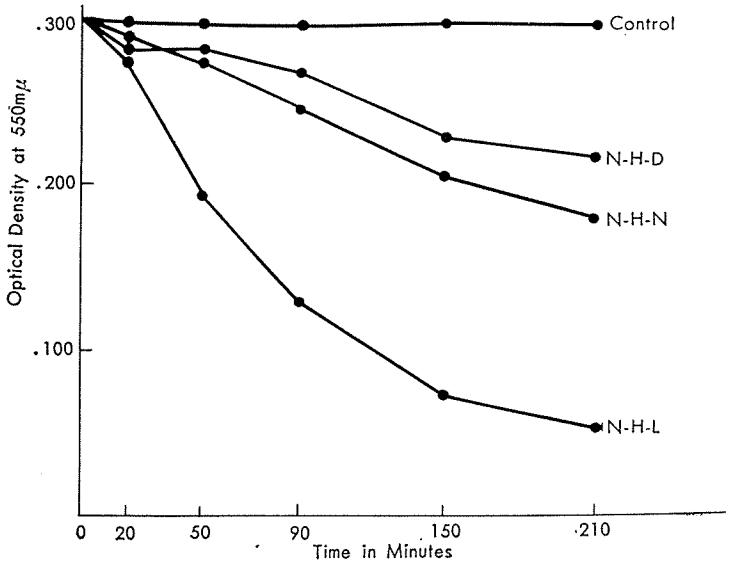

Fig. 1 Bacteriolytic Activities of Various N-H Extracts against *Salm. choleraesuis var.* Kunzendorf 5210

To elucidate the specificity of the increased bactericidal action of N-H-L occuring on immunization, the activity of N-H-L was tested against *Salm. enteritidis* (strain 1891) and *Salm. abortusequi* (strain β 202) (Figs. 3 and 4). The cells of both strains were also very susceptible to N-H-L and less susceptible to N-H-L. These facts suggest that the increased bactericidal action has no relationship to the antigenic specificity of somatic O antigen. Similar results have already been

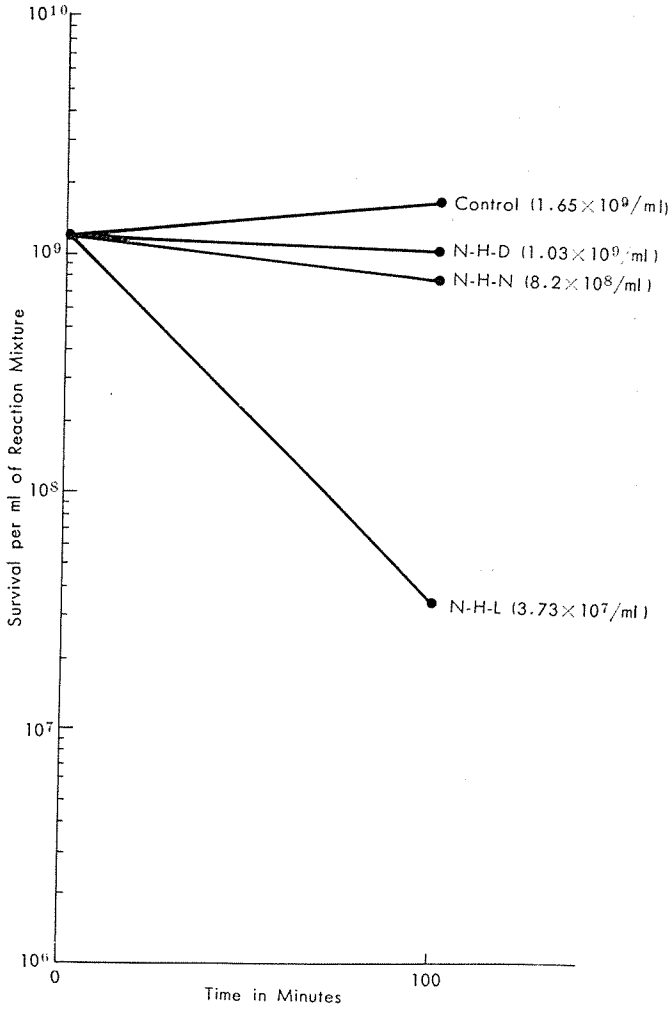

Fig. 2 Bactericidal Activities of Various N-H Extracts against *Salm. choleraesuis var.* Kunzendorf 5210

reported by Sato *et al.* (1961) in studies on intraphagocytic bacteriolysis in relation to the immunity evoked by live salmonella vaccine (*S. enteritidis*).

Although the nature of the active substances in N-H-L has not been elucidated, lysozyme can not account for the activity, because N-H-L, N-H-D and N-H-N were almost completely devoid of lysozyme. Histone, which was extracted from leucocytes by Hirsch *et al.* (1960), might be a causative agent. However, it is unknown whether the increased bactericidal and bacteriolytic activity in N-H-L is due to an increase in extractable histone.

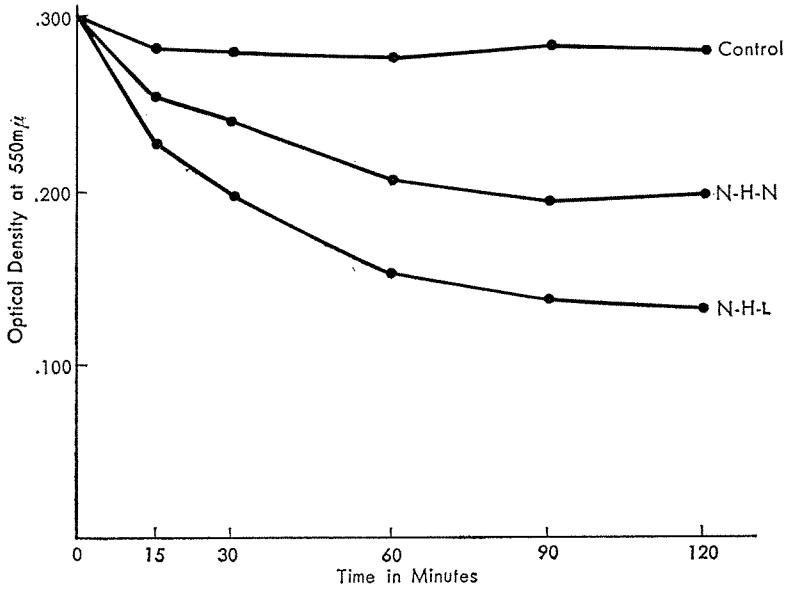

Fig. 3 Bacteriolytic Activities of Various N-H Extracts against *Salm. enteritidis* (strain 1891)

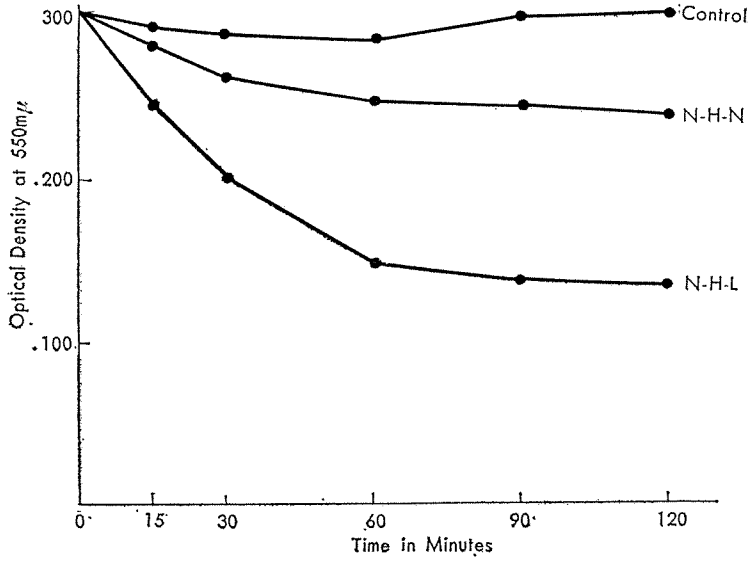

Fig. 4 Bacteriolytic Activities of various N-H Extracts against *Salm. abortusequi* (Strain β202)

REFERENCES

Amano, T. (1961). On the defence mechanisms against infection. *The Final Report* No. 2 (DA-92-557-FEC-31897).

Amano, T. (1962). On the defence mechanisms against infection. *The Final Report* No. 1 (DA-92-557-FEC-35672).

Hirsch, J.G. (1960). Further studies on preparation and properties of phagocytin. *J. Exptl. Med.* **111,** 323-337.

Oshima, S., Myrvik, Q. N. and Leake, E. (1961). The demonstration of lysozyme as a dominant tuberculostatic factor in extracts of granulomatous lungs. *Brit. J. Exptl. Pathol.* **42,** 138-144.

Sato, I., Tanaka, T., Saito, K. and Mitsuhashi, S. (1961). Studies on the experimental typhoid. *Jap. J. Bacteriol.* **16,** 877-886.

Skarnes, R.C. and Watson, D.W. (1957). Antimicrobial factors of normal tissues and fluids. *Bacteriol. Rev.* **21,** 273-294.

TAKASHI KURIMURA
SHUZO KASHIBA
TSUNEHISA AMANO

Department of Immunology,
The Research Institute for Microbial Diseases,
Osaka University, Osaka
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