climated mice longer than from unacclimated mice is not understood. Perhaps the decreased number of lymphocytes in donor skin (and therefore in the registration of antigenicity) is responsible for the increased transplant survival observed in Group III.

Since is is known that hypoxia raises the level of circulating adrenal corticoids, the involution of the thymus in acclimated mice is probably mediated through the increased activity of the pituitary-adrenal axis. Hamer and Krohn(6) showed that ACTH injections lead to a prolonged retention of skin homotransplants in rats, and Finkel(7) demonstrated a close correspondence between spleen size and antibody production. Both of these studies suggest a relationship between the functional state of the thymus and spleen and the immunological potential of the animal.

On the basis of the above results it is postulated that the stress associated with exposure to reduced barometric pressure depletes the lymphoid elements of the animal to such an extent that a longer time is required for initiation of transplant rejection. Studies are under way to determine more precisely the manner in which altitude acclimation affects the immune response of mice to foreign antigens and skin homotransplants.

Summary. Prior acclimation of either or both the donor and host to reduced barometric pressure prolongs the retention of skin homotransplants in mice. Spleen and thymus studies suggest that the increased tolerance of acclimated mice to skin homotransplants may be due to an alteration of the animal's immunological state.

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Sero-protection Against Intracerebral *Salmonella typhosa* Infection in Chicks.* (29759)

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In attempts to assay experimentally the immunizing potency of typhoid vaccines, one of the methods commonly used is to vaccinate humans or laboratory animals and to measure the capacity of their sera passively to protect mice against fatal infection (or development of bacteremia and tissue invasion) with *S. typhosa*. Among institutions concerned with this problem, the details of testing procedure have differed. Thus, antiserum has been given intraperitoneally, intravenously, intramuscularly or subcutaneously; the time interval between injection of serum and the subsequent bacterial inoculum has been varied, from 30 minutes to 48 hours; most investigators have administered the challenge dose intraperitoneally, using large numbers of living bacilli suspended in saline or fewer organisms incorporated in suitable preparations of hog gastric mucin. The disadvantages of the respective methods have been extensively discussed in the literature (1-4) but there is still no general agreement as to which procedure is preferable; this is particularly true in regard to the mode of infection.

The intracerebral route of inoculation with

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TABLE I. Passive Protection of Chicks by Antiserum Given 24 Hours Prior to Intracerebral Challenge with *S. Typhosa.*

Challenge strain	Inoculum (viables)	Pre-treatment of chicks	Fatality	Terminal cultures* from Brain	Spleen	Cloaca
Ty 2	20	None	6/6†	5/6‡	6/6	0/6
	200	"	3/4	3/4	3/4	1/4
	"	.2 ml normal serum	5/6	6/6	6/6	0/6
	"	.2 " immune "	0/9	0/9	0/9	0/9
Watt	17	None	4/6	5/6	5/6	0/5
	170	"	7/9	8/9	5/9	3/9
	"	.2 ml normal serum	6/9	9/9	7/9	1/9
	"	.2 " immune "	1/9	0/9	0/9	0/9

* Cultures made at autopsy, following spontaneous death or sacrifice at termination of experiment.

†No. dying during period of observation/No. inoculated.

‡ No. of birds yielding positive cultures from organ/No. examined.

S. typhosa was introduced by Norton and Dingle(5) who found that thereby fatal infection of mice could be initiated with relatively small numbers of a freshly isolated strain and that rabbit antiserum, administered intraperitoneally either before or simultaneously with the challenge, could confer protection against 2-10 M.L.D. Their results were confirmed by Landy *et al*(6) who also demonstrated the activity of mouse antiserum in such tests. In evaluating rabbit antisera prepared with vaccines used in the field study of human typhoid in Osijek, Yugoslavia, Standfast likewise employed the intracerebral method of challenge but did not find it advantageous when comparing results with those he obtained by intraperitoneal challenge(7).

It has been observed that young chicks are highly susceptible to intracerebral inoculation with *S. typhosa*, as few as 2 viable organisms sufficing to initiate fatal disease(8). It therefore seemed worthwhile to ascertain whether passive protection could be demonstrated advantageously in this model of infection. It was decided to employ chicken antisera at the outset, in order to observe the action of antibodies derived from the homologous animal species.

Materials and methods. The *S. typhosa* strains used had been tested previously for ability to infect chicks when given in small numbers by the intracranial route. The birds, of various breeds, were hatched in our laboratory or were obtained from commercial sources immediately after hatching; they were fed on antibiotic-free mash. The procedures for handling the chicks as well as for maintaining, inoculating and recovering the bacterial strains have been described(8). "Normal" serum was collected from adult chickens, some of which were reared in the laboratory. Antiserum was obtained from chickens which over a period of 3½ weeks were injected on 6 occasions with a total of 1.2×10^{10} acetone-killed and -dried cells of the Ty 2 strain; the organisms were derived from infected allantoic fluids of the 12th or 13th egg passage.† The sera were stored at 4°C without preservative, until needed. Pools of several sera which contained both anti-Vi (titers 1:40-1:80) and anti-O (titers 1:10-1:80) agglutinins were made for use in the passive protection tests; each chick received 0.2 ml of undiluted or diluted pooled serum intramuscularly, divided equally in each thigh.

Results. In a preliminary trial, groups of 7- or 8-day-old birds received undiluted serum from normal or immunized chickens and were challenged 24 hours later with organisms of the Ty 2 or Watt strains (Table I). Death occurred in most of the control animals given 17-200 viables but no serum, after a delay of 4-8 days; in chicks less than 4 days old at the time of challenge such fatalities would have been expected to occur 1-2 days post-inoculation(8). *S. typhosa* was usually recovered from the brain and/or spleen, less frequently from other viscera

† We are indebted to Dr. William O. Owings for preparing the vaccines and antisera at our suggestion.

TABLE II. Sterilization of Infection by Antiserum Given at Varying Intervals in Relation to Intracerebral Challenge with 80-300 Viables *S. typhosa* Ty 2.

Exp	Treatment of chicks	Terminal cultures after days of infection 1	2	3	4	6–13
A	Antiserum (dil. 1:10) – 3 hr before challenge	2/3*	0/3	0/3	0/3	—
	" " " – at time of "	3/3	2/3	1/3	2/3	—
	" " " – 3 hr after "	5/5	2/3	1/3	1/1	—
	Normal serum (undil.) – " " before "	2/2	1/1	—	3/3	—
	No serum	1/1	2/2	—	5/5	—
B	Antiserum (dil. 1:10) – 24 hr before challenge	0/3	—	—	—	1/7
	" " " – 6 " " "	1/3	—	—	—	0/7
	" " " – 3 " " "	2/3	—	—	—	0/7
	Normal serum (undil.) – 24 " " "	3/3	—	—	—	5/7
	No serum	3/3	—	—	—	7/7

* No. of birds yielding *S. typhosa* from brain/No. examined.

(heart's blood, liver, lung, gall bladder, cloaca) which were also regularly cultured. "Normal" serum showed no evidence of ability to protect against fatal disseminated infection and the few surviving birds in the group also yielded positive cultures when sacrificed. In contrast, among birds given antiserum there were virtually no deaths during the 8-day period of observation and not a single positive culture was isolated from any animal.

On further testing of the pooled antiserum for potency, it was found that chicks given 1:10 dilution likewise yielded no *S. typhosa* whereas birds given 1:100 dilution were still infected when terminal cultures were made 10 days after challenge with approximately 30-100 viables of the Herbert, Panama 58 or Ty 2 strains. The 1:10 dilution was therefore employed in 2 experiments (Table II) wherein an attempt was made to ascertain the effect of varying the interval between challenge and administration of antiserum. In these trials, birds of the more resistant Vantress Cross breed were employed so that fatalities were infrequent but cultures from the brain and other organs of animals sacrificed or dying spontaneously provided evidence of the state of infection. As expected, control animals which were untreated or given normal chicken serum were found almost uniformly infected whenever examined. Results similar to these were obtained in birds which received antiserum either immediately or 3 hours after challenge; the infections were not regularly sterilized within 4 days. When antiserum was given 3-6 hours prior to challenge, organisms could be recovered from the brain on the following day but not thereafter; in chicks receiving antiserum 24 hours beforehand the infection was usually sterilized within 24 hours of challenge.

Discussion. The data presented here indicate that the intracerebral infection of chicks with *S. typhosa* does present advantages for analysis of certain aspects of the immunology of experimental typhoid. Since by the intracerebral route the bacterial inoculum need be only a very few viables, one can obviate those effects of endotoxin and other bacterial products which immediately complicate the situation when large numbers of organisms must be injected parenterally. It must be admitted that in chicks it may not be too feasible to study factors involved in active immunity since even initially highly susceptible, young birds become naturally much more resistant during the period when the development of antibodies would be expected following vaccination(8). However, sero-protection tests can be readily performed with the conditions adjusted so that for unprotected animals the end result is usually either fatality (within a shorter or longer interval) or non-fatal infection, but in any event includes a bacteremic distribution and survival of organisms in various organs. Under such circumstances the prior administration of appropriate antiserum derived from chickens confers passive immunity, just as in mice certain antibodies from the homologous animal species or from rabbits, humans and horses have been shown to be active(3-7,9-18).

In the experimental system which we have

described the protection involves both life-saving effectiveness and also sterilization of the intracranial infection, with consequent prevention of hematogenous dissemination and persistence of bacteria in other sites. In mice the infection has sometimes been observed to continue in tissues for considerable periods, despite the ability of antiserum to prevent fatality when given prophylactically (10,12). Some contribution to the protective mechanism may be made by complement, which is demonstrable in the serum of chicks around the time of hatching; however, the antiserum used in our experiments had been stored beforehand for a year at 4°C so it probably was not an additional source of complement. It appears that in order to accomplish prompt sterilization of infection in the chick, antibodies must circulate and be able to cross the blood-brain barrier so as to attain an adequate level locally before the intracranial injection of *S. typhosa*. This requirement is influenced markedly by the potency of the antiserum as well as the time interval prior to the microbial challenge, judging from the results of our trials. If the organisms are introduced in the absence of a sufficient local concentration of antibody they may become facultatively intracellular parasites and hence resistant to eradication even though antibody accumulates in the local area later. Using a technic similar to that employed here, Gaines *et al*(19) obtained protection when antiserum was given to mice 1 hour beforehand. Norton and Dingle(5) noted that when the injection of antiserum was delayed until 5 hours after challenge, the life-saving benefits in mice were reduced as compared with those obtained when the serum was given 2-5 hours before, or simultaneously with, the intracerebral bacterial inoculum.

No attempt has been made to evaluate here the role of the respective antibodies directed against various antigenic constituents in strains of *S. typhosa*; this will be done elsewhere. It is recognized that there are undoubtedly other factors which contribute significantly to resistance in typhoid, in addition to circulating antibody. Since the passive immunity test in the chick permits one to measure the protective effects of the serum component with a considerable degree of sensitivity, however, further work should be done to determine and improve the value of the method so that it can perhaps be used with human and other animal antisera.

Summary. Young chicks were protected against intracerebral infection with *S. typhosa* by the prior intramuscular injection of chicken antiserum containing anti-Vi and anti-O agglutinins. Passive immunity was manifested by prevention of death and by sterilization of the tissues. To achieve this end it was necessary to administer the antiserum in quantity and at a time sufficiently in advance of the challenge such as to ensure an adequate concentration of antibody at the intracranial site when the microorganisms were introduced.

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Relationship of 5′-Nucleotidase Activity in Lamb Muscle to Selenium Levels Administered to their Dams.* (29760)

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White muscle disease (WMD) of sheep is a selenium responsive malady common to many areas of the world with an inherent deficiency of selenium in the forage. It has been previously reported(2) that lambs afflicted with this myopathy exhibit a marked elevation of 5′-nucleotidase activity in semitendinosus muscle tissue. Selenium administered to pregnant ewes will protect the subsequent lambs from developing WMD. To determine what the relationship of selenium to 5′-nucleotidase might be, muscle tissue specimens from lambs of both selenium treated and untreated dams were assayed for 5′-nucleotidase.

Methods and materials. The semitendinosus muscle tissue samples to be assayed were selected from lambs 6 weeks of age whose dams had been randomly allocated to experimental treatments as follows:

Lot 1—Control alfalfa hay (Union, Oregon) plus ¼ lb oats

Lot 2—Basal alfalfa hay (Madras, Oregon) plus ¼ lb oats

Lot 3—Basal alfalfa hay plus 0.1 ppm selenium with respect to the total ration in ¼ lb oats

Lot 4—Basal alfalfa hay plus ¼ lb oats plus 30 mg selenium in 10 ml water, given orally as a single dose

Lot 5—Basal alfalfa hay plus ¼ lb oats plus 30 mg selenium in wax parenterally as a single dose.

Selenium was administered as sodium selenite. The control hay (Lot 1) contained about 0.07 ppm, the basal hay 0.02 ppm and the oats 0.01 ppm selenium. The incidence of both gross and microscopic WMD lesions was nil in lambs from all lots except Lot 2, where 7 of 17 lambs developed lesions(1).

The semitendinosus muscle samples were obtained from freshly slaughtered lambs at 6 weeks of age, packaged in polyethylene bags, immediately frozen and stored for approximately 1 year at —20°C before being assayed. Six samples of muscle per treatment group were selected randomly, but equally distributed between sexes, except Lot 2 where afflicted lambs only were chosen.

The enzyme extract was initially prepared by transferring approximately 2 g of frozen muscle to a tissue press (Howard Apparatus Co., Inc., Dover, Mass.) with 5 ml of 0.05 M NaCl - 0.005 M Tris buffer, pH 8.3 and pressed to remove the bulk of the connective tissue. The macerated material less the connective tissue was transferred to a Potter-Elvehjem homogenizer and the residue pressed again with an additional 5 ml NaCl-Tris. The total pressed material was homogenized at 0-5°C in about 10 ml NaCl-Tris for 2 minutes with 1 minute rest time between minutes to prevent frictional heating and the smooth homogenate quantitatively transferred into polyethylene cups. The homogenate was centrifuged at 5,400 × *g* for 15 minutes at 0-5°C and the supernatant diluted to 10 × the weight of the tissue (10% tissue weight equivalent). Five ml of this enzyme extract was dialyzed overnight against two 125 ml portions of the NaCl-Tris. The remainder of the extract designated "Fresh" was retained for immediate use.

The assay procedure used by Schubert *et al* (2) was modified in that relative amounts

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