EXPERIMENTAL RUNT DISEASE IN MICE CAUSED BY SALMONELLA TYPHIMURIUM, VAR. COPENHAGEN*, ‡

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Considerable theoretical interest has been aroused by the controversy as to whether immunological runting may be passed within an isologous mouse strain. Siskind *et al.* (1) and Jutila and Weiser (2) observed runting when they injected neonates with spleen cell suspensions from isologous runts, whereas both Simonsen (3) and Dineen (4) were unable to duplicate these findings. The successful passage of runting reported in this paper would appear to support those protagonists who claim that runting can be serially passaged.

Runting was also used in the present work as a measure to determine the validity of the F_1 hybrid law which states that an F_1 hybrid does not mount an immunological reaction against donor tissues from either parental strain. Recent results casting doubt upon this law have utilized indirect experimental assay methods involving appraisal of the fate of cellular grafts injected into preimmunized and irradiated hosts (5, 6). Therefore, although Billingham and Brent (7) and later Jutila and Weiser (2) had not observed runting when parental neonatal mice were injected with spleen cell suspensions obtained from their F_1 hybrids, I repeated their experiments and did indeed observe runting.

These results, the successful passage of runting and the runting of parental neonates when injected with immunologically competent cells from hybrids, raised the possibility, ever present, that the observed runting was not due to an immunological reaction, but to an infectious agent. And indeed, in both studies, it was possible to isolate from the spleens of some runts, albeit it with difficulty, a strain of *Salmonella typhimurium*, var. *copenhagen*.[1] Pure cultures of this organism with or without the addition of "clean"[2] spleen suspensions, when

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[1] This identification was confirmed by Dr. F. Kauffmann, Statens Serum Institut, Copenhagen.

[2] A "clean" spleen suspension is a suspension from a mouse newly arrived in the laboratory, not injected with *S. typhimurium*, and not showing splenomegaly or hepatomegaly.

injected into isologous or homologous neonatal mice resulted in the occurrence of the signs and symptoms used by most workers as the basis for runting. Some of these parameters are splenomegaly (8, 9), hepatomegaly (9), failure of the young animal to gain weight (10), or death (2, 9).

Materials and Methods

Animals.—Inbred and F_1 hybrid mice of the following strains obtained from the Roscoe B. Jackson Memorial Laboratory were used throughout the study: A/J, DBA/2J, C3H/HeJ, CBA/J, C57BL/6, B6AF$_1$/J, CAF$_1$/J, B6DF$_1$/J, and C32DF$_1$/J. Neonates were bred in our own animal farm, usually by matings of inbred parents, and only first generation progeny was used.

Cell Suspensions.—Spleens were removed from donor mice after cervical dislocation and cell suspensions prepared by the method of Martinez *et al.* (11) in which the spleen tips are cut and splenic pulp expressed by gentle pressure with a bent probe. The cells were suspended in about 2 ml of Hanks' balanced salt solution (BSS) and the suspension gently expressed one or two times through 22, 25, and finally 27 gauge needles. This crude cellular suspension was next spun at 500 *g* for 5 minutes and the supernatant fluid was discarded. The deposit was suspended in approximately 1 ml of BSS per spleen and was now ready for injection. All operations were done at room temperature.

The majority of injections of neonatal recipients was intravenous through the orbital branch of the anterior facial vein using a tuberculin syringe and 30 gauge ½ inch needle. Some animals were injected subcutaneously with the same size needle, the injection being just below the eye, and still others were injected intraperitoneally by passing a 30 gauge 1 inch needle through the triceps muscles in the pectoral region from whence it was pushed posteriorly through the skin until its tip passed the lower margin of the liver, after which it was passed through the body wall into the peritoneal cavity. The volume injected was usually 0.05 ml. Cell counts were done after transfer using 0.1 per cent trypan blue to determine the number of viable cells. There were usually 120 to 180 $\times$ 10^6 nucleated cells per spleen.

Bacteriology.—Heavy suspensions of the specimens being examined for *S. typhimurium* were plated on thickly poured plates of MacConkey's and *Salmonella-Shigella* agar and were also inoculated into selenite-F enrichment broth. After 8 to 10 hours in the broth, aliquots were spread heavily over MacConkey and *Salmonella-Shigella* plates. The plates were examined after both 24 and 48 hours' incubation at 37°C and suspicious non-lactose-fermenting colonies were subcultured onto Kligler's triple iron-agar. Putative *S. typhimurium* organisms were then tested with *S. typhimurium* O and H antisera by slide agglutination.

RESULTS

Preliminary Experiments.—These preliminary experiments were designed to determine if runting could be serially passaged and if the injection of parental strains with spleen cell suspensions from F_1 hybrids would result in runting. In both sets of experiments mice were injected intravenously on the 1st day of life with approximately 6 to 10 million viable spleen cells.

In these first experiments a runt is defined as a sickly emaciated mouse, failing to gain weight, and usually dying between the 5th and 30th days of its life. At postmortem some degree of splenomegaly is observed. Deaths occurring before the 5th day are not included, except in those experiments on the serial passage of runting.

To study the serial passage of runting A/J, CBA/J, or $B6AF_1/J$ mice were injected with C57BL/6 cells. As will be seen in Table I runting was very common. Serial passages were attempted by making suspensions of the spleens of one or more obviously sick animals and injecting these suspensions in the customary manner into recipients isologous with the original recipients. Runting was noted in the first passage and could be transferred a second time. The severity of runting, as judged by total mortality and rapidity of death, was increased on passage.

TABLE I

Serial Passage of "Runting"

Strain combination	Passage	Runts/No. injected	Day after injection on which count made
C57BL/6 → CBA/J	—	7/7	19
	1st	4/4	19
	2nd	10/10	1*
C57BL/6 → CBA/J	—	7/7	19
	1st	3/8	10
	2nd	10/10	3*
C57BL/6 → CBA/J	1st	3/8	10
	2nd	9/9	11
C57BL/6 → A/J	—	10/10	11
	1st	5/5	5*
C57BL/6 → $B6AF_1/J$	—	8/8	48
	1st	5/5	9

Approximately 6 to 10 × 10^6 spleen cells injected intravenously on day of birth. A "runt" is a sickly emaciated mouse, failing to gain weight and usually dying between the 5th and 30th days of life. Some splenomegaly is usually observed.

* All dead.

Meanwhile the capacity of hybrid cells to react against parental tissue was studied. The results in Table II show that of 6 strain combinations used, runting occurred much more frequently in the $B6AF_1/J$ to C57BL/6 combination than in all others, and it was therefore this combination that was later chosen for subsequent detailed study.

The immunological basis of the runting and death observed until now appeared questionable and the following experiment increased this uncertainty. A C57BL/6 runt that had been injected subcutaneously rather than intravenously was found to have a dry abscess just below the inoculation site. Suspensions were made of the spleen and the liver of this animal and these were injected subcutaneously into a mixed group of isologous and homologous animals varying in age from 1 to 4 days. All died. Determined efforts were made to culture bacteria from the abscess, spleen, and liver of the C57BL/6 donor.

From both abscess and spleen, a bacterium, subsequently characterized as *S. typhimurium*, var. *copenhagen*, was isolated in pure culture.

This organism, either alone, or added to spleen cell suspensions, was used subsequently in these studies on runting. In addition, both "infected spleen suspensions" and "clean spleen suspensions" were used. The former were from mice, injected or not with *S. typhimurium*, but showing grossly enlarged spleens and livers. The latter refers to suspensions from mice which had very recently arrived from Bar Harbor, had not been injected with *S. typhimurium*, and did not have splenomegaly or hepatomegaly.

In the remainder of the experiments to be described in this work, a runt is defined not only as sickly emaciated animal failing to gain weight and usually

TABLE II

Incidence of Runting When F_1 Hybrid Cells Injected into Parental Neonates

Strain combination	No. of litters	Runts/No. injected
B6AF_1/J → A/J	4	2/19
C32DF_1/J → DBA/2J	7	1/30
C32DF_1/J→ C3H/HeJ	4	0/20
CAF_1/J → A/J	10	5/51
B6AF_1/J → C57BL/6	12	21/69
B6D2F_1/J → C57BL/6	4	1/25

Approximately 6 to 10 × 10^6 spleen cells injected intravenously on day of birth. A runt is a sickly emaciated mouse, failing to gain weight and usually dying between the 5th and 30th days of life. Some splenomegaly is usually observed.

dying between the 5th and 30th day of its life, but as an animal which at postmortem exhibits a grossly enlarged reddened fibrous spleen and an enlarged liver with areas of necrosis. Even if mice died as early as 24 hours after injection, but not before, and if there was no sign of maternal neglect, they were counted as runts, except in those instances in which physically altered cell suspensions were used.

Factors Influencing the Incidence of Runting.—The age of the neonate and the size of the inoculum have both been shown to play a part in the development of runting. Both these variables were therefore tested with pure cultures of *S. typhimurium.*

Tenfold log dilutions of a pure culture of *S. typhimurium* were injected intraperitoneally into 1-day-old C57BL/6 mice. The results (Table III) show that runting due to S. *typhimurium* is dose-dependent.

C57BL/6 mice of different ages were now injected intraperitoneally with *S. typhimurium*, and it will be observed (Table IV) that runting could occur when the mice were injected as late as the 8th day postpartum but older mice did

not runt. Although it is difficult to compare the results of injections on different days because of the variation of the dose, the incidence of runting did decline when older mice were used.

Physical Treatment of Inocula.—Various methods are available to destroy the viability of animal cells and thus prove that their integrity is necessary for the induction of tolerance (although see Martinez *et al.*, reference 12) and the

TABLE III

Influence of Dose of S. typhimurium on Runting of C57BL/6 Neonates

No. of organisms	7	70	700	7000
No. of mice	4	4	4	6
Days till death, average	19	7	4	3

Mice injected intraperitoneally on 1st day of birth. All mice dead at 30 days.

TABLE IV

Runting Incidence When S. typhimurium Injected into C57BL/6 Mice at Various Times after Birth

Age of mice when injected	Dead/Total	Dead
days		*per cent*
1	33/33	100
2	12/13	92
3	19/24	79
4	10/17	60
5	11/15	73
6	Not done	Not done
7	3/14	21
8	4/11	36
9	0/12	0

Experiment terminated at 30 days. The dosage was not constant but was betweeen 10^3 and 10^4 organisms per mouse given intraperitoneally.

development of runting. The most commonly used method is the alternate rapid freezing and thawing of suspensions.

Spleen cell suspensions from $B6AF_1/J$ mice known to be carrying *S. typhimurium* and spleen cell suspensions from clean animals were frozen and thawed 4 successive times by alternately placing in a dry-ice–butanol mixture and a 37°C water bath. An overnight broth culture of *S. typhimurium* was diluted one hundredfold in BSS and treated in the same way. Counts of the *S. typhimurium* suspension before and after treatment showed a reduction of 99.9 per cent.

C57BL/6 neonates were injected intraperitoneally with these spleen suspensions. From the results in Table V it will be observed that babies which received the frozen-thawed cells survived longer than those which received the

untreated cells. None of the animals which received cells of clean $B6AF_1/J$ donors showed the features of runting.

Sonication, heat, or x-irradiation at selected dosages will destroy the integrity of animal cells, and yet only reduce, sometimes by as little as a few per cent, the number of viable bacteria. Therefore if runting is due to infection, rather than to an immunological reaction on the part of the graft against the hosts, the treatment of infected spleen cell suspensions by any one of these methods

TABLE V

Effect of Different Physical Treatments of Spleen Cell Suspensions on Runting of C57BL/6 Neonates

Treatment	Days until death		
	Exp. 1	Exp. 2	Exp. 3
Freeze-thaw	8, 9, 2 live	16, 16, 2 live	17, 22, 2 live
Control	5, 5, 8, 10	16, 16	16, 22
Heat 48.5°C/20 min	6, 7, 7	10, 13	7, 9, 9
Control	6, 6	6, 6, 6, 10	8, 10, 10
Sonication/ 30 sec	7, 9, 9, 10		
Control	10, 10, 12		
3000 r to donor	1, 1, 1, 1, 1, 1	4, 4, 4, 5, 6, 6	14, 15, 18*
Suspension	16, 22, 16, 16	8, 9, 16, 16	10, 17, 20
Supernatant after 500 *g*/4 min	5, 20, 20, 1 lives		
Deposit after 500 *g*/4 min		9, 16, 20, 20	16, 17, 20, 21

Experiment terminated at 30 days.

* This animal killed on day 18 and showed all symptoms of runting.

should not reduce the incidence of runting. If, on the contrary, runting is due to an immunological reaction, these treatments should be efficacious.

C57BL/6 neonates were injected intraperitoneally with untreated, sonicated (30 seconds), or heated (48.5°C for 20 minutes) spleen cell suspensions, or with spleen cell suspensions from lethally irradiated mice (3000 roentgens). It will be observed (Table V) that in no instance did treatment reduce the incidence of runting. The very early deaths sometimes observed when treated cells were injected might be explained on the basis of endotoxin being released from these cells; however, this seems unlikely since it was not observed when suspensions of clean animals were used. More likely the protection afforded the *S. typhimurium* by the cells of the suspension is removed and the liberated microorganisms are free to colonize the recipient.

Further experiments were done in which a spleen cell suspension of $B6AF_1/J$ mice, before being centrifuged at 500 *g* for 4 minutes, was divided into 2 portions. One was not centrifuged; the other was centrifuged in the customary manner, the supernatant fluid kept, and the deposit made up to the original volume in BSS. Three inocula were thus available. C56BL/6 neonates were injected intraperitoneally. The results recorded in Table V show that deaths occurred with all 3 types of incoula. The supernatant fluid contains very few if any spleen cells and should not cause runting if an immunological reaction is necessary. If, on the other hand, runting is due to infection, the slow speed of centrifugation will not pack down all the free *S. typhimurium* and runting might occur. This indeed was observed. (A caution must be inserted here. Billingham and Brent, reference 7, showed that spleen cell suspensions which were not lightly centrifuged often caused the immediate death of neonates due to toxic debris; however, the first death was not observed until the 5th day postinoculation.)

Attempts to Prevent Runting by Immunization.—Although there is great doubt as to the efficacy of active immunization of mice against salmonellosis it seemed of interest, especially because of reports that runting had been prevented by immunization with spleen cells isologous with the donor suspensions (9, 10, 13), to attempt to immunize potential mothers with *S. typhimurium*. In addition, the effect of passive serum treatment was studied. Reports on the value of this treatment are even less encouraging than those on active immunization.

Several C57BL/6 breeders received 3 intraperitoneal 0.75 ml injections at 4-day intervals of a saline suspension of a *S. typhimurium* H and O vaccine. Other breeders were given 1 injection in the hindfoot-pads and the flanks with the same saline suspension mixed in incomplete Freund's adjuvant. Eleven normal male C57BL/6 mice were injected with the saline vaccine and were bled 2 weeks after the last injection; the sera were collected and used for passive protection tests.

The 1-day babies of 5 immunized breeders, 2 of which had received the saline vaccine and 3 the Freund's vaccine, were injected intraperitoneally with a spleen cell suspension from $B6AF_1/J$ mice to which had been added *S. typhimurium*.

The results in Table VI show that whereas the control animals developed signs and symptoms of runting terminating in death, most of the babies of the vaccinated breeders appeared healthy and survived.

In other experiments neonates received intraperitoneal injections of $B6AF_1/J$ cells mixed with *S. typhimurium*, and antiserum was given intraperitoneally at the times shown in Table VI to some of the litter. Those animals which received the antiserum lived longer than those which did not. It is possible to speculate that if the passive antiserum treatment had been prolonged additional protection could have been afforded these neonates.

Thus, contrary to expectations, active and passive immunization with this strain of *S. typhimurium* did protect against runting and salmonellosis.

Effect of Adult Isologous Cells on Runting.—In other laboratories (2, 7, 9, 13) it has been possible to prevent runting by the injection of adult spleen cells isologous for the neonate together with homologous cells. Experiments were therefore done in which isologous adult spleen cells were added to either spleen

cell suspensions from contaminated B6AF$_1$/J mice or to pure cultures of *S. typhimurium*. These mixed suspensions were then injected into day-old C57BL/6 mice. In no instance was the incidence of runting diminished.

Bacteriology.—Clearly, an inapparent infection with *S. typhimurium* might be present in the C57BL/6 colony and it therefore seemed desirable to study the epidemiology of this organism within the colony.

TABLE VI

Active and Passive Immunization of C57BL/6 Mice against Runting Caused by S. typhimurium, var. copenhagen

Treatment	No. organisms injected	Days until death	Mean till death
			days
0.1 ml antiserum i.p.* days 2, 4, 6	310	18, 18, 19, 19	18.5
0.1 ml saline i.p. days 2, 4, 6	301	6, 7	6.5
0.1 ml antiserum i.p.* days 2, 4, 6	310	14, 17, 17, 17	16
0.1 ml saline i.p. days 2, 4, 6	310	5, 6	5.5
0.05 ml antiserum i.p.* days 1, 2, 5	14,500	5, 7, 9, 9, 13, 13	9
0.05 ml saline i.p. days 1, 2, 5	14,500	5, 5, 7	6
0.05 ml antiserum i.p.* days 1–8	1000	12, 21, 21, 28, 30, 1 lives	>21
0.05 ml saline i.p. days 1–8	1000	6, 7	6
Freund's to mother‡	5000	6/6 live	
Freund's to mother	14,000	7/8 live	
Freund's to mother	5000	15, 16, 18, 18, 19, 20	18
H and O vaccine to mother§	2250	4/4 live	
H and O vaccine to mother	5000	5/5 live	

Experiment terminated at 30 days.

* The antiserum was prepared by immunizing male C57BL/6 with an H and O vaccine of *S. typhimurium*, var. *copenhagen*.

‡ Breeders were immunized once with the H and O vaccine mixed with incomplete Freund's adjuvant.

§ Breeders were immunized 3 times at 4-day intervals with the H and O vaccine.

Fecal and blood specimens of 30 adult C57BL/6 mice which had been injected when babies with pure cultures of *S. typhimurium* or known infected spleen cell suspensions, and of 29 adult mice which, although not actively infected, had shared cages since birth with the injected animals, were cultured. All samples were inoculated on *Salmonella-Shigella* and MacConkey agar both before and after enrichment in selenite-F broth.

S. typhimurium was isolated from the feces of 8 animals which had received contaminated material, whereas in no instance was a positive isolate obtained from uninoculated littermates. Fecal specimens from 26 mice, 9 injected and 17 uninjected, which had failed to yield any *Salmonella* were taken a 2nd and 3rd time, at approximately 2-week intervals, but from only 1 of the injected mice was a positive culture now obtained. Blood samples were always negative.

Seventeen mice were killed and their spleen suspensions cultured in the same manner as blood and fecal specimens. Six of these failed to yield *S. typhimurium*, although the animals were known to have been injected as neonates with the organism and autopsy showed enlarged reddened fibrous spleens.

Twelve mice of 6 to 8 weeks of age, which had been injected with *S. typhimurium* when neonates, were bled and their sera collected. Agglutination tests of their sera with H and O antigens of *S. typhimurium*, starting with a serum dilution of 1:40, were all negative.

DISCUSSION

In spite of repeated failures to demonstrate that runting is due to infection a vague doubt still exists (14, 15), not that postmortem infection occurs, which it surely does, but that bacteria or viruses are the *ipso facto* cause of runting. Billingham and Brent's (7) original description of runting was stringent and included signs, symptoms, and autopsy findings. Later workers (1, 2, 8, 9) stressed some, but not all, of these parameters and introduced other methods for measuring runting; in general, this has resulted in a less rigorous definition.

In the experiments reported in this paper, failure to gain weight, splenomegaly and hepatomegaly associated with areas of necrosis, and usually death within 30 days, subsequent to injection of inocula into neonatal mice, were taken as the criteria of runting. On the basis of these criteria runting was observed not only with living spleen cell suspensions but also with suspensions poor in immunologically competent cells (liver suspensions), with isologous and homologous suspensions, and indeed even with pure cultures of *S. typhimurium*, injected with or without spleen cells. The injections could be intravenous, intraperitoneal, or even subcutaneous. Runting produced by such a variety of inocula and by various routes has been reported only on rare occasions by other workers (2, 9).

The size of the inoculum and the age of the recipient were both important factors in the development of the runting due to *Salmonella* reported here. Runting still occurred with as few as 7 organisms (the fewest used) but the smaller the inoculum, the longer the life of the mouse. The incidence of the disease decreased, although not in a steady manner, the older the recipient became, until when neonates of 9 days were injected, they did not runt. Similar dose–life span and age-susceptibility relationships have been reported when homologous spleen cells suspensions are injected into neonates (2, 9, 16).

The disruption of viable spleen cells by physical methods to prove that the integrity of these cells is necessary for runting may, depending on the conditions used, be a two-edged weapon, destroying not only the spleen cells, but also bacteria which might be present. Thus, a frozen-thawed suspension of spleen cells, with or without *S. typhimurium*, did not produce runting, because both cells and bacteria had been destroyed. On the other hand, sonication for

30 seconds or heating at 48.5°C for 20 minutes of spleen cell suspensions with added *S. typhimurium*, or the *in vivo* lethal irradiation of mice containing *S. typhimurium*, result in preparations which still produce runting because the treatment is efficacious in destroying only the spleen cells, but permits some of the bacteria to survive. Then again, the speed of centrifugation of macerated spleen cell suspensions containing *S. typhimurium* will influence the result obtained when the supernatnat fluids are injected into neonatal mice. Low-speed centrifugation will leave a supernatant fluid, rich in bacteria, which will cause runting; high-speed centrifugation will bring down the bacteria, and the supernatant fluid when injected into neonates will not cause runting.

The passive protection afforded against *Salmonella* runting by antisera made against an O and H vaccine of *S. typhimurium* and the active immunization of pregnant females with this same vaccine are a little surprising. Most workers are doubtful of the protection which active and certainly passive immunization will offer against *Salmonella* in mice (17). Siskind and Thomas (9) were also able to protect against runting by actively immunizing pregnant females with spleen cell suspensions. The latter result was confirmed by Russell (13) after his initial attempts had failed (10), and in addition he was able to prepare protective antisera using skin transplants as antigens. If contaminating organisms are present in a cellular vaccine they, as well as the cells, may be immunogenetic, but it is difficult to invoke such a suggestion when skin transplants are the antigenic stimulus.

All attempts to abrogate *Salmonella* runting by the injection of adult isogenic spleen cells simultaneously with the *Salmonella* failed, an experimental finding which sharply differs from the results (2, 7, 9, 13) observed in studies on runting. This may represent a useful method of distinguishing runting due to *Salmonella* from immunological runting.

The fact that members of the mouse colony were harboring *S. typhimurium* without any outward symptoms and that actively infected mice did not infect litter mates sharing the same cage might ellicit some surprise. The literature, however, has many examples of such a situation (18), and indeed a strain exhibiting just such characteristics of high virulence and low infectivity was described by Topley *et al.* (19). It must be stressed that isolation of the organism from infected animals was not always easy; further, repeated feces cultures from the same animal did not always yield the offending organism.

Strain differences are known to play a role in resistance to infection with *S. typhimurium* (20–22) and it has also been suggested that they may play a part in runting (2). In the publications of Billingham and Brent (7), Siskind and Thomas (9), and Jutila and Weiser (2) runting was observed most frequently with derivatives of the C57 mouse.

The presence of *S. typhimurium* in my mouse colony has clouded the answer to the two polemics I set out to clarify, namely, can runting be passed within

an isogenic strain and can the F_1 hybrid mount an immunological reaction against parental strains. In both examples positive results were obtained but these need not necessarily be the result of immunological injury due to graft *versus* host reaction, but could be the product of the offending *Salmonella*. Studies with accredited pathogen-free mice might resolve these problems. However, it may be stated categorically that pure cultures of *S. typhimurium*, var. *copenhagen* can cause many of the signs and symptoms and postmortem findings now frequently accepted as the parameters of runting. Are we therefore to believe that all runting described in the literature is the result of infection and not of an immunological reaction?

The original report of Billingham and Brent (7) certainly, with the exception of 3 to 5 mice, seems to have as its basis the immunological reactivity of the graft against the host; more questionable are the papers of Siskind *et al.* (1, 9, 23). The 3, or possibly 5 mice, which Billingham and Brent mentioned, died several days after skin grafting and had very large spleens some of which "superficially resembled those of mice suffering from leukemia" and hypertrophied lymph nodes. Passage of the spleens into young hybrids was without effect but one wonders the age of the recipients. Dingle (24) describes mice in experimental salmonellosis as having enlarged livers and spleens, the former having small yellow pin-head sized lesions which increase in size and become necrotic.

It is important that stricter attention be paid to the parameters used to judge graft *versus* host reactions and that autopsy be performed whenever possible. Involution of lymphoid tissues would appear to be a critical point for distinguishing between true immunological runting and salmonellosis. A shortened half-life of host erythrocytes and specific antibodies against them might also be helpful although here too it must be noted that both of these can occur as a result of non-specific causes (25–27). Finally, Dutton showed that 8 of 10 organisms, including *S. typhimurium*, were more virulent when injected subcutaneously or intraperitoneally than when injected intravenously (28). Therefore, if possible, one should inject some members of the litter subcutaneously using heated cells and others should be injected intravenously with non-heated cells. Only the latter should exhibit true immunological runting.

SUMMARY

The strain of *Salmonella typhimurium* isolated from the subcutaneous abscess of a runted mouse and used in this study was somewhat unusual, but not unique, in that it had a high virulence for young mice, yet low infectivity. This strain could mimic many of the features, signs, and symptoms of immunological runting when injected into neonates, either in pure culture, or when mixed with spleen cells, or when present in infected isologous or F_1 hy-

brid spleen cells. Thus, the incidence of *Salmonella* runting was dose-dependent and related to the age of the neonate. Runts failed to gain weight, were sickly, and usually died within 30 days. They had a marked splenomegaly and hepatomegaly associated with areas of necrosis. However, in marked contrast to immunological runts they did not have lymphoid atrophy.

The incidence of runting was diminished when frozen-thawed spleen cell suspensions were used, but not with sonicated or heated suspensions or spleen cells from lethally irradiated mice. Runting could be prevented by immunizing breeders with *S. typhimurium*, and serum from mice immunized against *S. typhimurium* protected neonates injected with this organism. Isologous adult spleen cells did not protect against *Salmonella* runting.

It is suggested that in studies on runting only the intravenous route be used and that heated cells serve as a control. More rigid criteria should be applied to runting than those frequently accepted and mice should be autopsied whenever possible.

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