STUDIES ON THE RUNTING SYNDROME IN NEWBORN MICE*

By GREGORY W. SISKIND,‡ M.D., AND LEWIS THOMAS, M.D.

(From the Departments of Medicine and Pathology, New York University College of Medicine, and the Third (NYU) Medical Division, Bellevue Hospital, New York)

(Received for publication, June 9, 1959)

It was found by Billingham and Brent (1), by Simonsen (2), and by Woodruff and Sparrow (3) that a considerable percentage of newborn mice, rats, and chicks which were treated with adult, homologous spleen cells, lymph node cells, buffy coat leucocytes (4), or thymus cells (4), after an initial period of apparently normal growth, became ill, exhibited a failure to grow at a normal rate, and died by 4 weeks of age. This disease has been referred to as the runting syndrome.

It has been reported that runted mice exhibit diarrhea (3, 4), an anemia with a positive Coombs test (2), splenomegaly (2, 5, 6), hepatomegaly (5, 6), and atrophic changes of their lymphoid tissue (1, 2, 4, 7). Billingham and his coworkers found that the incidence of runting in mice varied with different strain combinations (4), being greater with greater genetic disparity between donor and recipient (4). They also found that the runted mice were tolerant of skin grafts from the original cell donor's strain (1, 4). Simonsen (2) found that blood from adult chicks produced splenic enlargement if given to chick embryos, while blood from newborn chicks was not active. Mechanical disruption of the cells in a Potter homogenizer destroyed their spleen-enlarging activity (2). Inoculation of parent strain newborn mice or chicks with spleen cells from the F^1 hybrid of that strain with some other strain failed to produce runting (4–6); while parent strain spleen cells produced runting of newborn F^1 hybrid animals (5).

Most workers in the field (1, 2) have considered the runting syndrome to be the result of an immunological reaction of the grafted spleen cells against a tolerant host.

It is the purpose of this paper to explore further certain aspects of the runting syndrome, including several immunological methods for protecting newborn mice against the development of the syndrome.

Materials and Methods

Mice.—The mice used in these experiments were, in most cases, DBA/2, A/Jax, C57Bl/6, Balb/c, CBA, C57Br, and CAF^1 inbred strains obtained from the Jackson Memorial Labora-

* This work was supported by grants from the United States Public Health Service, B-1127 and H-2022, and from the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-590.

‡ Work performed during tenure of a Medical Student Research Fellowship.

tory, Bar Harbor, Maine, and maintained on Wayne Lab blox mouse pellets and water *ad libitum*. In some experiments Swiss Webster newborn mice were employed.

Cell Donors.—The cell donors were 6 to 10 week old inbred mice of the strains mentioned above, housed in cages containing ten mice per cage, and provided with food and water as described above.

Cell Recipients.—The cell recipients were newborn mice of the strains mentioned above. Newborn mice were obtained either from pregnant mice supplied by the Jackson Memorial Laboratory or from our own colony of breeders established with breeding stock obtained from the Jackson Memorial Laboratory. Breeding mice were housed three mice (two females and one male) per cage and supplied with food and water *ad libitum*. Each cage was examined once daily for newborn animals. After treatment, newborn mice were returned to and left with their own mothers.

Spleen Cell Preparations.—Donor mice were sacrificed by cervical dislocation, spleens were removed intact, and passed through a garlic press. The mash was then suspended, by vigorous pipetting, in sterile normal saline so that the material from three spleens was suspended in 1 ml. of saline. Each newborn mouse, unless otherwise stated in the text, received 0.05 ml. of this suspension intraperitoneally. Cell counts were performed on randomly selected preparations and proved to be fairly constant, with each newborn mouse receiving between eight and twelve million nucleated spleen cells.

Cell suspensions of other organs (liver, kidney, and mesenteric lymph node) were prepared in a similar manner.

Injection of Newborn Mice.—All injections of newborn mice were given *via* the intraperitoneal route. To avoid leakage, a 27 or 30 gauge needle was employed and the needle was passed through the animal's thigh muscle and into the peritoneal cavity. Injections of foreign cells, unless otherwise stated, were always given within 24 hours after birth.

Preparation of Serum.—Anti-C57B1/6 serum was prepared by immunizing 6 to 8 week old DBA/2 male mice with adult C57B1/6 spleen cells. Recipients received initial intraperitoneal injections of 0.5 ml. of saline suspension containing two-thirds of a C57B1/6 spleen per ml. Two weeks later, each received approximately 0.1 ml. of an emulsion, prepared from a suspension containing three C57B1/6 spleens per ml. mixed with an equal volume of Difco complete bacto-adjuvant (containing an added 50 mg. of Difco dried *Mycobacterium butyricum* per 10 ml. Difco complete adjuvant), into the four toepads. One week later, each mouse received a second similar injection of C57B1/6 cells with complete adjuvant into the toepads. The mice were bled, by incision of the retroorbital plexus, 1 week after the final immunization. The blood was allowed to clot and cell-free serum was prepared by centrifuging twice at 2500 R.P.M. for 20 minutes.

Serum Treatment.—Newborn mice treated with serum received daily intraperitoneal injections of serum, starting 2 days after birth, according to the following dosage schedule:

No. of days after birth	Ml. of serum
2	0.02
3	0.05
4	0.07
5	0.10
6	0.10
7	0.10
8	0.10
9	0.10

Criterion for Runting.—The runting syndrome as seen in newborn mice receiving homologous spleen or lymph node cells consists of failure to grow at a normal rate, diarrhea, anemia, focal coagulative necrotic liver lesions, splenomegaly, and death within 2 to 3 weeks after birth. All of the above occur in various combinations in runted mice. Because of the simplicity of measurement, sharpness of endpoint, and reliability, we chose death of the mouse between 6 and 30 days after birth as our criterion for runting. This criterion generally included all mice which would be considered runts according to the other possible criteria mentioned above. Deaths occurring during the first 5 days after birth were regarded as consequent upon the administration of a relatively large volume of fluid to a newborn mouse; and mice dying during this period were not considered in calculations of runting incidence reported in this paper. Mice surviving 30 days in almost all cases were of normal size and appeared healthy.

EXPERIMENTAL

Factors Influencing Runting Syndrome.—To determine what types of tissues, other than homologous spleen cells, would produce runting, newborn mice were

TABLE I

Effect of Spleen and Liver Cells Given to Mice within 24 Hours after Birth

Type of tissue		Source	Incidence of death between 6 and 30 days of age
	Lymph node	Homologous	18/25
Litter mate controls	Liver	Homologous	2/16
	Spleen	Homologous	11/14
	No treatment		1/17
Litter mate controls (Balb/c)	Spleen	Homologous	8/8
	Spleen	Isologous	2*/28
	No treatment		0/9

* These two animals died 20 and 26 days after birth and were of normal size and did not show liver lesions on autopsy.

treated with saline suspensions of homologous liver cells, homologous kidney cells, homologous mesenteric lymph node cells, and isologous spleen cells within 24 hours after birth. As indicated in Table I, homologous adult mesenteric lymph node cell suspensions also caused runting.

Litters of newborn mice were divided into three groups: one group received homologous liver cell suspensions; the second group received homologous spleen cell suspensions; and the third group was left untreated. As indicated in Table I, the animals which had received homologous spleen cells became runted, while animals which had received homologous liver cells were unaffected. The weights of animals which had received homologous liver cells were approximately equal to those of their litter mate controls.

Twenty-four Swiss Webster newborn mice received C57Br kidney cell

suspensions. Three died during the first 3 days after birth. The remaining twenty-one appeared normal. Twelve were sacrificed for blood studies between 10 and 25 days after birth. None showed any abnormalities on autopsy and all had normal red blood cell counts. Nine were allowed to grow to maturity. These were alive and well 40 days after birth, when they were sacrificed, and showed no lesions on autopsy.

Litters of Balb/c mice were randomly divided into three groups: one group received C57B1/6 spleen cells; the second group received Balb/c adult female spleen cells; and the third group was untreated. As indicated in Table I, isologous spleen cells failed to produce runting.

The effect of different conditions of administration of homologous spleen

TABLE II

Incidence of Runting with Various Strain Combinations of Mice

Donor strain	Recipient strain	Incidence of runting
C57B1/6	A/Jax	78/79 (99%)
C57B1/6	CAF[1]	32/32 (100%)
C57B1/6	DBA/2	78/85 (92%)
C57B1/6	Balb/c	38/41 (93%)
C57B1/6	Swiss Webster	33/40 (82%)
C57B1/6	CBA	16/16 (100%)
C57Br	DBA/2	14/14 (100%)
CBA	A/Jax	9/20 (45%)
Balb/c	DBA/2	4/16 (25%)
A/Jax	CBA	17/22 (77%)

cells upon the incidence of runting was studied. It was found, as shown in Table II, that the incidence of runting depends upon the particular combination of donor and recipient strains employed. Spleen cell preparations were made, and injections performed as described in the methods section.

Newborn mice were treated with one-fifth the usual dose of spleen cells within 24 hours after birth. As shown in Table III, the incidence of runting decreased as one decreased the dose of homologous spleen cells administered.

Mice were given homologous spleen cell suspensions, prepared and administered as indicated in the methods section, at different times after birth. As shown in Table IV, the incidence of runting decreased as the time interval between birth and the administration of the homologous spleen cells increased. Little runting is seen when the spleen cell injection is delayed more than 4 days after birth.

Liver Lesions in Runted Mice.—A characteristic lesion of liver necrosis was frequently observed in mice suffering from the runting syndrome. In the gross, the lesion consisted of patchy, white areas located predominantly along the

free edge of the liver. Microscopically, the lesion was a focal area of coagulative necrosis associated with little or no cellular inflammatory reaction. The lesions were generally of subcapsular distribution, although some centrally located lesions were seen.

TABLE III

Influence of Dosage of Spleen Cells in Incidence of Runting

Donor strain	Recipient strain	Dosage of spleen cell suspension*	
		0.05 ml.	0.01 ml.
C57B1/6	DBA/2	78/85 (92%)	15/26 (58%)
C57B1/6	A/Jax	78/79 (99%)	2/6 (33%)
C57B1/6	Swiss Webster	33/40 (82%)	7/50 (14%)

* Spleen cell suspension prepared with three adult mouse spleens per ml.

TABLE IV

Runting Incidence with Spleen Cell Injection at Various Times after Birth

Donor strain	Recipient strain	Age of mice when injected					
		1*	2	3	4	5	6
C57B1/6	Balb/c	38/41 (93%)	19/24 (79%)	5/10 (50%)			
C57B1/6	A/Jax	78/79 (99%)	12/14 (86%)		7/14 (50%)		
C57B1/6	CAF[1]	32/32 (100%)	15/24 (62%)	13/14 (93%)		6/16 (38%)	0/11
C57B1/6	DBA/2	78/85 (92%)	23/31 (74%)	4/11 (36%)	5/20 (25%)	6/25 (24%)	
C57B1/6	Swiss Webster	33/40 (82%)	6/16 (37%)	8/75 (11%)	12/68 (18%)		

* 1 = day of birth.

To determine the incidence of the liver lesion in mice receiving homologous spleen, DBA/2 and A/Jax newborn mice were treated with C57B1/6 spleen as described in the methods section. A/Jax mice were sacrificed between 11 and 14 days of age and DBA/2 mice were sacrificed when 9 to 10 days of age. All mice were examined for the presence of gross liver lesions.

Sixty-five out of seventy-two, or 90 per cent, of the treated A/Jax mice were found to have liver lesions visible in the gross, of the type described

above. Twenty-nine out of thirty-seven, or 78 per cent, of treated DBA/2 mice had similar lesions.

Anemia in Runted Mice.—Newborn mice were treated with homologous spleen, homologous liver, or isologous spleen, as described in the methods section. At various times after birth, the animals were bled from the retroorbital plexus and white and red blood cell counts performed.

It was found, as shown in Table V, that many of the runted mice had anemia of variable degree, with red cell counts averaging less than 3 million during the first 10 days after injection of spleen cells. The white blood cell counts ranged from leukopenia as low as 250, to leucocytosis as high as 21,700. Much greater variability was seen in the white blood cell counts of runted mice, than in those of normal mice or of mice which had received homologous liver

TABLE V

Averages of Red Blood Cell Counts on Mice Treated with Homologous and Isologous Tissues within 24 Hours after Birth

Age	Untreated	Received homologous spleen	Received homologous kidney	Received isologous spleen
days				
5–10	5.14* (18)‡	2.93 (6)	4.90 (2)	—
11–16	6.38 (37)	4.87 (21)	6.43 (4)	6.04 (6)
17–22	7.90 (8)	6.10 (4)	8.69 (2)	8.50 (3)
23–25	8.21 (4)	—	8.18 (5)	—

* Numbers indicate red cell counts, expressed as millions of cells per cubic milliliter.

‡ Figure in parenthesis indicates number of mice upon which counts were done.

or isologous spleen. Mice which had received homologous liver or isologous spleen showed normal red blood cell and white blood cell counts.

Necessity for Living Cells to Produce Runting.—Several experiments reported below were designed to determine whether viable cells were required to produce the runting syndrome and to rule out the possibility of an infectious agent as the cause of runting.

Spleen cell suspensions were prepared as indicated in the methods section. Half of each suspension was then subjected, three times, to rapid freezing and thawing, while the other half was left untreated. Each litter of mice was divided randomly into two groups: one received the frozen-thawed spleen cell suspension, while the other received the untreated spleen cell suspension. DBA/2, A/Jax, and CBA mice were used as recipients. C57B1/6 mice were used as spleen cell donors. Out of fifteen mice receiving untreated spleen cells, thirteen or 87 per cent became runted, while of twenty mice receiving frozen-thawed spleen, only two or 10 per cent died and these two were of normal size and had no liver lesions when autopsied.

Suspensions containing six C57B1/6 spleens per ml. of saline were prepared. The suspensions were homogenized in a Potter homogenizer for 10 to 15 minutes. Each of nine newborn A/Jax mice received 0.05 ml. of this homogenate within 24 hours after birth. These mice grew normally, were sacrificed after 15 days, and examined in the gross and microscopically for liver lesions and splenomegaly. No abnormalities were observed.

C57B1/6 spleens were ground with a mortar and pestle and then suspended in saline so as to have suspensions of 6 spleens per ml. These suspensions were centrifuged at 2,500 R.P.M. for 30 minutes. Each newborn mouse received 0.05 ml. of the cell-free supernatant within 24 hours after birth. Of the twenty-two mice so treated, two died when 11 days old. The remaining twenty mice appeared normal and were sacrificed 15 days after birth. No liver lesions or splenic enlargement was found in any of these mice, either in the gross or microscopically.

Since spleen cells which had been subjected to freezing and thawing or homogenization failed to cause runting, as did also the cell-free extracts of cells, it seems reasonable to conclude that viable, intact cells are necessary to produce the syndrome.

Protection against Runting Syndrome.—Prevention by treatment with isologous adult spleen cell suspensions: It was thought that isologous adult spleen cells, by providing the newborn mouse with the ability to react immunologically against foreign tissues, might protect it from runting.

All animals in each of several litters received homologous spleen as previously described. The litters were then randomly divided: one group received no further treatment; the other group received 0.05 ml. of a suspension of 4 isologous spleens per ml. of saline, intraperitoneally, within 30 minutes following the injection of homologous spleen. Other litters were divided into three groups: the first group received no further treatment; the second group received isologous spleen, as described above; and the third group received isologous liver. The liver cell suspensions were prepared so as to be equivalent in concentration with the isologous spleen cell suspensions on a wet weight basis. A number of litters were divided into two groups: one group received no further treatment; the other group received isologous liver cell suspensions as above. Balb/c, CBA, A/Jax, and DBA/2 mice were used as recipients. C57B1/6 mice were used as spleen donors.

Our experimental results, shown in Table VI, indicate that isologous spleen cells given to newborn mice within 30 minutes following injection of homologous spleen cells conferred significant protection against the runting syndrome. None of the twenty-three mice which received only homologous spleen cells survived 30 days, whereas thirteen out of twenty-one, or 62 per cent, of the mice treated with isologous spleen cells survived and were well at that time. Those mice which died despite treatment with isologous spleen cells tended to

live longer than did their untreated litter mates. The data further show (see Table VI) that isologous adult liver cells, given within ½-hour following injection of homologous spleen cells, conferred no protection against runting. All of twenty mice treated with isologous liver cells died before 20 days of age.

It was necessary that treatment with isologous spleen cells be instituted soon after the injection of homologous cells in order to prevent runting. When the injection of isologous cells was delayed until 24 hours after birth, no protection was demonstrable (Table VI).

Protection against Runting by Antiserum against the Homologous Strain.—It was next considered that newborn animals which had received foreign spleen

TABLE VI

Protection by Adult Isologous Spleen Cells against Runting Syndrome When Given within 30 Minutes after Homologous Spleen

Group	No. of mice	No. of mice dying					Surviving 30 days
		Day of death					
		6–10	11–15	16–20	21–25	26–30	
Newborn mice given homologous spleen cells	47	12	27	6	0	2	0
Newborn mice given homologous spleen and isologous spleen 30 min. later	21	0	0	4	4	0	13
Newborn mice given homologous spleen and isologous liver 30 min. later	20	5	12	3	0	0	0
Newborn mice given homologous spleen and isologous spleen 1 day later	19	3	11	3	0	0	2

cells might be protected against runting by treatment with serum prepared from mice immunized against the spleen cell donor's strain.

In these experiments, newborn DBA/2 mice were used as recipients and adult C57B1/6 mice as spleen cell donors. Anti-C57B1/6 serum was prepared in DBA/2 adult mice as indicated in the methods section. All mice were given homologous spleen, as previously described, and then each litter was randomly divided: one group was treated with normal DBA/2 serum; the second group was treated with DBA/2 anti-C57B1/6 serum. In some litters, a third group which received only homologous spleen was included. Animals were treated with serum daily, from 2 days through 9 days after birth, as indicated in the methods section.

The results, summarized in Table VII, indicate that the "anti-donor strain" serum was effective in protecting animals receiving homologous spleen from becoming runted. Normal mouse serum provided no protection against runting.

Protection against Runting by Immunizing Mothers against the Homologous

Cell Donor.—In view of the finding that serum from immunized mice could protect against runting, it was considered that immunization of mothers against the spleen cell donor strain, prior to parturition, might, as a result of passage of antibody from the mother to the offspring, protect the newborn against runting.

One group of experiments was performed using Balb/c mothers and C57B1/6 spleen cell donors. The Balb/c females were immunized against C57B1/6 mice by means of repeated intraperitoneal injections of C57B1/6 spleen cell suspensions in saline. Animals born to these mothers, mated with normal Balb/c males, were treated with C57B1/6 spleen as described previously. As shown in Table VIII, in the offspring of two of the treated mothers, we observed no decrease in the incidence of runting as compared with the offspring of untreated mothers, whereas, in the case of two other treated mothers, a marked decrease was observed in the incidence of runting (from 93 per cent with offspring of untreated mothers to 25 and 20 per cent with offspring of immunized mothers).

TABLE VII

Protection by Anti-Serum against Homologous Spleen Cells

DBA/2 litter No.	Incidence of runting*		
	Received normal DBA/2 serum	Received DBA/2 anti-C57B1/6 serum	No serum treatment
1	1/2	0/2	1/1
2	2/2	0/2	—
3	1/1	1/2	—
4	3/3	1/4	3/3
5	4/4	0/3	2/3
6	1/1	0/2	—
7	2/2	3/3	—
Total.....	14/15	5/18	6/7

* All received C57B1/6 spleen cells on day of birth.

A second series of experiments was carried out, using A/Jax mothers and C57B1/6 spleen cell donors. The mothers were immunized against C57B1/6 mice by repeated inoculation with C57B1/6 spleen cell suspensions in complete Freund's adjuvant into the toepads. The offspring of these females mated with normal A/Jax males, were treated with C57B1/6 spleen cell suspensions as described above. The data from these experiments, as shown in Table VIII, indicate that immunization of A/Jax mothers regularly reduced the incidence of runting from the 99 per cent normally seen with this strain combination to 20 per cent.

DISCUSSION

It has been suggested by previous workers (1, 2) that the runting syndrome is the result of an immunological reaction of the foreign cells against a tolerant host. The data presented here are consistent with this hypothesis.

The cells which produce runting are derived from lymphoid tissues (spleen or lymph node), generally assumed to be involved in antibody formation. Liver and kidney cells in comparable concentrations are not capable of causing runting. The donor must be genetically different from the recipient; isologous spleen does not cause runting.

The decrease in the incidence of runting which was observed to occur as the interval between birth and the time of administration of homologous spleen

TABLE VIII

Incidence of Runting in Litters from Mothers Immunized against Homologous Spleen Cells

Recipient strain and treatment	Breeding cage No.	Incidence of runting
Balb/c mothers immunized against C57B1/6	I	11/12 (92%)
Balb/c mothers immunized against C57B1/6	II	3/12 (25%)
Balb/c mothers immunized against C57B1/6	III	2/10 (20%)
Balb/c mothers immunized against C57B1/6	IV	9/10 (90%)
Balb/c mothers untreated		38/41 (93%)
A/Jax mothers immunized against C57B1/6	V	4/20 (20%)
	VI	
	VII	
	VIII	
A/Jax mothers untreated		78/79 (99%)

cells was increased is of interest in view of a similar decrease in the induction of skin graft tolerance, reported by Billingham and Brent (1). This parallelism suggests that tolerance may be necessary in order to permit the foreign spleen cells to survive long enough to cause runting. The failure of frozen-thawed cells, homogenized cells, and cell-free supernatant from ground spleen suspensions to cause runting, indicates the necessity of viable, intact cells in the syndrome, and tends to exclude the possibility that runting is due to the transmission of an infectious agent from the spleen donor to the newborn animal. Such procedures as freezing and thawing, and homogenizing would be expected to destroy the immunological reactivity of cells, but it is unlikely that any known bacteria or viruses would be completely eliminated by these measures. Similar results with homogenized cells have been reported previously by Simonsen (2).

The etiology of the focal, coagulative necrotic, liver lesions described in the

runted mice is obscure. The pathological appearance is consistent with an ischemic origin. The possibility that the lesion is of an infectious nature resulting from seeding of the liver with organisms arising in the intestines cannot be ruled out. Infections would not be unlikely in animals as debilitated as the runted animals become. Two other possible causes for these lesions must be considered: (*a*) vascular occlusion due to agglutination of red blood cells as a result of the action of antibodies formed by the foreign spleen cells, and (*b*) direct immunological reaction of the foreign cells against the liver tissue. To the vascular occlusion hypothesis one can raise the objections that infarcts of the liver are very unusual, and that if this were the actual pathogenesis, one would expect to find thrombotic lesions in other organs in addition to the liver and such lesions have not been found. No evidence can be offered for or against the hypothesis of a direct immunological reaction of the foreign cells against the host liver tissue being the origin of the lesions observed.

It was shown by Billingham, Brent, and Medawar (8) that tolerance can be eliminated by treating the tolerant animal with adult isologous lymph node cells. The finding that runting can be prevented by treatment of the mice with isologous adult spleen cells may indicate an analogous situation. The efficacy of this procedure in protecting against runting may lie in its ability to prevent the animal from becoming "tolerant." That is, by providing it with adult, immunologically competent cells, capable of reacting against the foreign spleen cells, the animal may be prevented from becoming "tolerant." If, as seems likely, tolerance is a prerequisite for runting, preventing the animal from becoming tolerant would inevitably protect it from runting. It is possible that by providing the newborn mouse with adult spleen cells, it is converted into an essentially immunologically mature state, in which it is unable to become tolerant of foreign tissue and so will not become runted. The failure of isologous liver cells, which are immunologically inactive, to protect against runting is consistent with this hypothesis. The failure of isologous spleen, administered 24 hours after the injection of homologous spleen, to protect against runting is a surprising finding. Possibly, the foreign spleen cells multiply sufficiently rapidly, so that, with 1 day's head start, they are able to achieve some sort of "privileged position" in which it is impossible to destroy them before they have sufficiently damaged the host to result in the latter's death.

The ability of immune serum, directed against the spleen cell donor strain, to protect against runting strongly suggests that some factor in the serum, presumably antibody, is able to destroy the foreign cells or render them immunologically inactive. The finding that offspring of immunized mothers are protected against runting when given spleen cells from the strain against which the mother had been immunized, is interpreted as indicating the passage, from mother to offspring, in the colostrum or across the placenta, of antibody directed

against the donor line of cells. Parenthetically, these observations furnish indirect evidence to support the theory that circulating antibody may be involved in the rejection of homografts.

SUMMARY AND CONCLUSION

Runting was produced by homologous spleen or lymph node cell suspensions, but not by isologous spleen or homologous liver or kidney cell suspensions.

The incidence of runting (*a*) varied with the particular strain combination employed, (*b*) increased with increased dose of foreign cells, and (*c*) decreased as the time interval between birth and inoculation with foreign cells was increased.

A focal, coagulative necrotic, liver lesion was described in runted mice.

It was found that viable cells were required to produce the runting syndrome. Frozen-thawed cells, homogenized cells, and the cell-free supernatant of ground spleen suspensions failed to produce runting.

Runted mice were found to have an anemia of variable degree, and white blood cell counts ranging from marked leukopenia to severe leucocytosis. Mice receiving isologous spleen or homologous liver or kidney showed normal red and white blood cell counts.

Isologous spleen cells, given to newborn mice within 30 minutes following injection of homologous spleen cells, conferred significant protection against the runting syndrome. Isologous spleen injected 1 day after the injection of homologous spleen failed to protect. Newborn mice which had received homologous spleen cells were protected from becoming runted by treatment with "anti-cell donor strain" serum. The offspring of mothers which had been immunized against the spleen cell donor's strain failed to become runted when treated with homologous spleen cells.

The data are regarded as compatible with the concept, presented by previous workers, that runting is the result of an immunological reaction of foreign cells against a tolerant host.

The authors would like to acknowledge the kind assistance of Dr. Chandler A. Stetson, Jr., Chairman of the Department of Pathology, New York University College of Medicine, and Dr. Philip Y. Paterson, Assistant Professor of Microbiology, New York University College of Medicine.

BIBLIOGRAPHY

1. Billingham, R. E., and Brent, L., A simple method for inducing tolerance to skin homografts in mice, *Transplant. Bull.*, 1957, **4**, 67.
2. Simonsen, M., The impact on the developing embryo and newborn animal of adult homologous cells, *Acta Path. and Microbiol. Scand.*, 1957, **40**, 480.
3. Woodruff, M. F. A., and Sparrow, M., Further observation on the induction of tolerance of skin homografts in rats, *Transplant. Bull.*, 1957, **4**, 157.

4. Billingham, R. E., Studies of the reaction of injected homologous lymphoid tissue cells against the host, *Ann. New York Acad. Sc.*, 1958, **73,** 782.
5. Cock, A. G., and Simonsen, M., Immunological attack on newborn chickens by injected adult cells, *Immunology*, 1958, **1,** 103.
6. Simonsen, M., Engelbreth-Holm, J., Jensen, E., and Poulsen, H., A study of the graft-versus host reaction in transplantation to embryoes, F1 hybrids and irradiated animals, *Ann. New York Acad. Sc.*, 1958, **73,** 834.
7. Egdahl, R. H., Roller, F. R., Swanson, R. L., and Varco, R. L., Acquired tolerance to homografts and heterografts in the rat, *Ann. New York Acad. Sc.*, 1958, **73,** 842.
8. Billingham, R. E., Brent, L., and Medawar, P. B., Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance, *Phil. Tr. Roy. Soc. London, Series B*, 1956, **239,** 357.