beyond the 50 to 70% found readily exchangeable, as well as the heterogeneity of the salivary tissue with regard to cell type, make compartmental analysis difficult with this system. In the absence of more extensive analysis it is not possible to estimate the magnitude of the potassium compartment in the slice which is affected by pilocarpine. Hence, no quantitative comparison of effects of pilocarpine on uptake and unloading of K in the slice and *in vivo* is appropriate. Nor can the mechanism of action of pilocarpine on the cell membrane be reliably deduced, although an increase in permeability would not be inconsistent with present data (11), especially if membrane potentials were not appreciably altered. Nonetheless, the data do give clear evidence that pilocarpine enhances uptake and loss of $K^{42}$ in slices of salivary gland tissue and that the effect of pilocarpine on loss of $K^{42}$ is inhibited by atropine. Thus, similarities are suggested in effects of stimulating agents on K-exchange *in vivo*(3) and in slices.

*Summary.* The presence of pilocarpine led to an increase in loss and uptake of $K^{42}$ in slices of rat submaxillary gland incubated under selected conditions. The influence of pilocarpine on the loss of $K^{42}$ was found to be inhibited in the presence of atropine. These effects on the exchange of $K^{42}$ in slices bear at least a qualitative resemblance to effects known to occur during stimulation of salivary glands *in vivo*.

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## Bacterial Infection and Wasting in Neonatally Thymectomized Rats.* (29383)

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It is generally accepted that in several strains of mice and in other mammals thymectomy in early life is associated with lymphopenia, wasting, and certain defective immunologic reactions(1-4). It is not clear whether these changes are specifically due to ablation of the thymus at a critical period of development of lymphoid organs or whether they represent secondary phenomena exaggerated by neonatal sepsis and, in some situations, selective breeding(5,6). Post-thymectomy wasting has been likened to runt disease or graft-versus-host reaction and several hypotheses have been formulated to explain its pathogenesis. It is the purpose of this paper to evaluate the significance of bacterial infection and wasting among adult survivors of neonatally operated rats.

*Methods.* Sprague-Dawley rats, all born between September 1963 and January 1964, were operated upon within 48 hours after birth. Anesthesia was achieved by cooling animals on an ice block covered with moistened paper towels. Four types of operations

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TABLE I. Incidence of Sepsis and Wasting in Neonatally Operated Rats.

| Treatment | Operation | Survivors 8 to 9 wk, No. & (%) | Sepsis in survivors, No. & (%) | | Wasting in survivors, No. & (%) |
|---|---|---|---|---|---|
| | | | Abscess, operation site | Peribronchitis & pneumonia | |
| Terramycin group | Thymectomy | 25 (22.1) | 3 (12.0) | 9 (36.0) | 2 ( 8.0) |
| | Thymus autograft | 21 (27.6) | 5 (23.8) | 5 (23.8) | 1 ( 4.8) |
| | Splenectomy | 25 (32.0) | 0 ( — ) | 6 (24.0) | 0 ( — ) |
| | Sham operation | 20 (41.6) | 1 ( 5.0) | 5 (25.0) | 0 ( — ) |
| Untreated group | Thymectomy | 17 ( 6.1) | 7 (41.2) | 10 (58.8) | 5 (29.4) |
| | Thymus autograft | 29 (17.4) | 7 (24.1) | 7 (24.1) | 1 ( 3.4) |
| | Splenectomy | 22 (18.2) | 0 ( — ) | 5 (22.7) | 1 ( 4.5) |
| | Sham operation | 24 (26.9) | 5 (20.8) | 6 (25.0) | 1 ( 4.2) |

were performed under clean but not aseptic conditions:

a) thymectomy

b) thymectomy, followed by immediate reimplantation of the thymus in the upper mediastinum

c) splenectomy

d) sham thymectomy or splenectomy

Two types of operations were performed within each litter. The rats were weaned at 4 weeks. Diet consisted of standard Rockland pellets. One group of litters was given water alone. The remainder was maintained on oxytetracycline HCl (Terramycin, Pfizer) in drinking water (6 g per liter) first given to the mothers and then to the weaned rats.

All animals were sacrificed and autopsied at 8 to 9 weeks of age. Tail blood was obtained for hematological and serum paper and immuno-electrophoretic studies prior to sacrificing. Total body weight and weight of various organs were recorded and histologic sections were made of lymphoid organs, bone marrow, liver, lung, kidneys, adrenals, as well as other sites when indicated. Specimens for bacteriological cultures were obtained from grossly infected tissues. In addition, a number of oxytetracycline-treated and untreated neonatally operated animals were immunized at 8 weeks with 3 successive intraperitoneal injections of 0.5 ml of horse serum. They were bled 7 to 10 days after the last injection and anti-horse serum precipitin reactions were tested on Ouchterlony agar-gel double diffusion plates. These studies will be presented in detail in another report.

*Results.* Survival rate, incidence of sepsis and wasting in neonatally operated rats are recorded in Table I.

*Survivors:* The immediate mortality rate following neonatal operations was negligible. However, as a combined result of post-operative morbidity, maternal cannibalism or neglect and intercurrent infections, the number of rats surviving 8 to 9 weeks after birth was considerably reduced, particularly among litters which were not treated with oxytetracycline. The percentage of survivors in the group of animals which underwent autografting of the thymus within the anterior superior mediastinum was considerably higher than that of thymectomized rats without thymic grafts. The effect of thymic grafts was limited, however, to animals which were not treated with oxytetracycline. The survival of sham-operated and splenectomized rats was significantly better than that of thymectomized litter mates.

Survival rates seem to follow seasonal variations. In our experience, animals operated upon during spring and summer survive better than those operated upon during late fall or winter. Furthermore, neonatal operations performed within 24 hours after birth, as in the case with most litters here, carry a considerably higher mortality rate than operations performed after 2 days of age.

*Sepsis in survivors:* Purulent exudation was noted at the site of skin incision in some animals as early as the 4th post-operative day. Thymectomy incision wounds tended to develop a purulent discharge or abscesses more frequently than splenectomy wounds. At autopsy, two main forms of sepsis were encountered: (1) Walled-off abscesses meas-

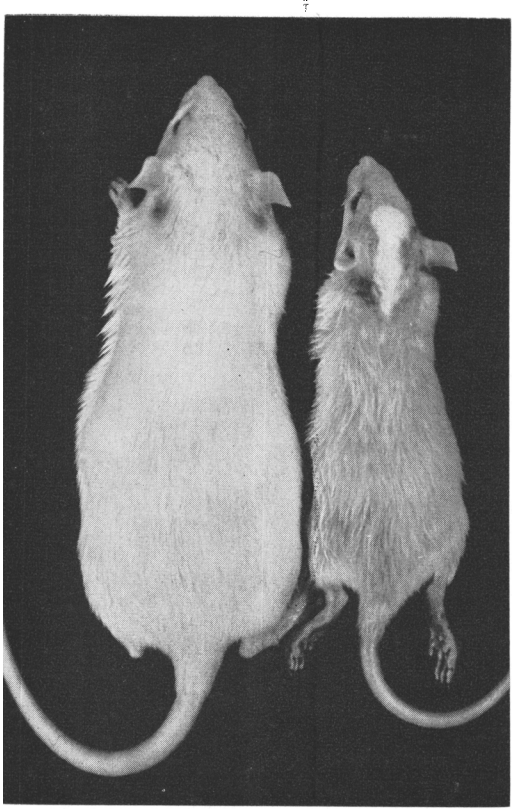

FIG. 1. Wasted male 8-week-old thymectomized rat compared to healthy sham-operated litter mate.

uring 1 mm to 2 cm in diameter, usually located along the upper anterior chest wall, region of the thymus and base of the neck. Such abscesses obviously denoted infection at the site of surgical procedure. (2) Bronchial and pulmonary inflammations, most frequently in the form of bronchiectasis with pus filling dilated bronchi, chronic peribronchitis, chronic pneumonia and lung abscesses. Occasionally, an acute hemorrhagic pneumonia was noted. The number of abscesses at the site of operations was considerably greater in thymectomized (including grafted) groups than in other operated animals (Table I). Administration of oxytetracycline was associated with a considerable reduction in number of wound infections, except in the group grafted with thymic tissue. It is possible that partial necrosis of the grafted thymus may have favored bacterial contamination. Bronchial and pneumonic lesions were encountered more frequently and in more extensive forms among thymectomized groups. The number of broncho-pulmonary lesions was not appreciably reduced by oxytetracycline therapy except in thymectomized groups.

*Wasting:* In this experiment, animals were considered wasted when their total body weight was equal to or was less than half the weight of healthy sham-operated litter mates of the same sex and age. In the absence of healthy survivors, an animal was considered wasted at 8 weeks of age if its weight was less than 125 g for a male and 100 g for a female. A typical example of wasting is illustrated in Fig. 1. Wasting was characterized by relatively sparse lusterless hair, weakness, high mortality rate as well as small stature. Wasted animals frequently developed labored breathing and had diarrhea on occasion. The onset of wasting was variable. It was noted as early as 2 weeks of age or as late as 6 weeks. There were no sex differences in the incidence of wasting. Wasted animals dying before 8 to 9 weeks frequently showed pneumonia or harbored abscesses in the mediastinum and anterior chest wall. In the group of animals sacrificed at 8 to 9 weeks (Table I), the incidence of wasting was considerably higher among thymectomized animals than in other operated groups. Furthermore, animals treated with oxytetracycline showed less tendency to wasting than animals given water alone.

A more detailed analysis of observations in wasted animals surviving 8 to 9 weeks after birth is presented in Table II. It is interesting to note that all wasted animals showed evidence of infection in the form of broncho-pulmonary inflammation (10 out of 11) or abscesses at the site of neonatal operation (4 out of 11). Cultures from grossly infected tissues yielded 3 main groups of organisms: diphtheroids, non-hemolytic streptococci and a Hemophilus species. As anticipated, lymphopenia was noted in wasted thymectomized animals as in non-wasted thymectomized rats. In most wasted rats, including thymectomized animals, there was a marked elevation of serum gamma globulin. In 4 out of 7 wasted thymectomized animals, serum gamma globulin concentration was significantly elevated above the level of that of healthy sham-operated or thymectomized rats. The

TABLE II. Observations in Wasted Rats.

| No. & sex | Group | Type of infection (bacteria cultured) | Total leucocytes & (lympho-cytes) mm³ | Serum gamma globulin, % |
|---|---|---|---|---|
| T 1 ♂ | Terramycin Thymectomy | Extensive bronchiectasis, peribronchitis and chronic pneumonia (No bacteria recovered) | 11,000 ( 4,838) | 38.7 |
| 67 A ♂ | *Idem* | Extensive bronchiectasis & peribronchitis Liver abscesses (No culture done) | 13,900 ( 6,116) | 13.8 |
| 64 A ♂ | Terramycin Thymus autograft | Extensive bronchiectasis & peribronchitis with lung abscesses (Hemophilus, ? species) | 10,050 ( 7,236) | 21.5 |
| 4 E ♀ | Untreated Thymectomy | Abscess, thymic region—1 cm (Diphtheroids, non-hem. streptococci) Extensive bronchiectasis & peribronchitis (Non-hem. streptococci) | 8,025 ( 4,333) | 29.3 |
| 10 B ♀ | *Idem* | Bilateral peribronchitis (Non-hem. streptococci) | 4,200* ( 3,108) | 9.6 |
| 31 G ♀ | " | Abscess, thymic region—0.4 cm (Diphtheroids) Chronic pneumonia, right upper lobe (Diphtheroids) | 10,250 ( 6,047) | 11.4 |
| 52 E ♂ | " | Abscess, thymic region—1 cm Chronic pneumonia with lung abscesses (Diphtheroids) | 10,750 ( 1,290) | 18.9 |
| 86 B ♂ | " | Extensive bronchiectasis, peribronchitis with lung abscesses (Hemophilus, ? species) | 6,600 ( 3,300) | 24.6 |
| 33 C ♀ | Untreated Thymus autograft | Chronic pneumonia with lung abscesses (Diphtheroids) | 21,850 (11,143) | 26.2 |
| 19 B ♀ | Untreated Splenectomy | Peribronchitis, right upper lobe Pericholangitis (No culture done) | 8,200 ( 3,772) | 21.7 |
| 48 E ♀ | Untreated sham splenectomy | Peritoneal & liver abscesses (No culture done) | 20,000 ( 9,800) | — |

* This count was done on a heart blood sample. Tail blood was insufficient.

highest elevation of serum gamma globulin ever noted in rats in this laboratory was seen in a wasted neonatally thymectomized animal with extensive broncho-pulmonary inflammation (Fig. 2).

The histologic appearance of the inflammatory lesions observed in wasted animals indicated a chronic process. The abscesses were all surrounded by a fibrous wall. The broncho-pulmonary lesions were also chronic. Although a purulent exudate was frequently present in dilated bronchi, cells present in peribronchial and pneumonic inflammatory reactions were predominantly plasma cells. These observations apply also to the inflammatory lesions of wasted neonatally thymectomized rats (Fig. 3). Lymph nodes in the immediate vicinity of inflammatory lesions were frequently infiltrated with numerous plasma cells. However, the spleens of wasted thymectomized animals tended to be atrophic and usually lacked well-formed Malpighian follicles as well as plasma cells. There was no histologic evidence of "autoimmune" or graft-versus-host reaction in the tissues of wasted animals.

*Precipitin reactions:* Results are shown in Table III. The percentage of positive anti-horse serum precipitin reactions was considerably higher in control sera than in those of thymectomized animals. However, sera of animals treated with oxytetracycline showed a significantly higher percentage of positive reactions. The percentage of reactors among

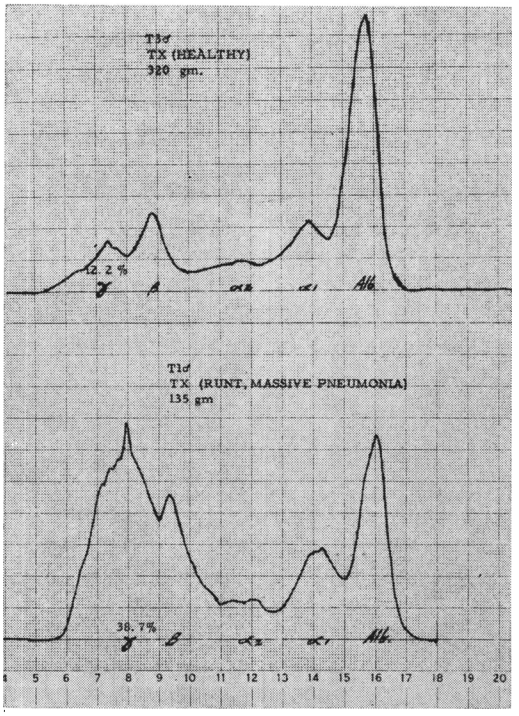


FIG. 2. Serum electrophoretic pattern of healthy thymectomized rat (top) and wasted thymectomized litter mate which developed massive bilateral pneumonia (bottom). Note the marked hypergammaglobulinemia of the wasted neonatally thymectomized animal.

oxytetracycline-treated thymectomized rats was approximately twice that of reactors among untreated thymectomized animals.

*Discussion.* The results clearly indicate a significantly higher incidence of bacterial infections, probably contracted early in life, as well as wasting in neonatally thymectomized rats than in other operated groups. In all instances of wasting reported here, there was evidence of chronic infection usually in the form of upper sternal or mediastinal abscesses or broncho-pneumonic inflammation. The incidence of sepsis and wasting was considerably reduced in rats treated with oxytetracycline. Furthermore, oxytetracycline-treated thymectomized and control animals showed a considerably more effective precipitin antibody response than untreated controls. All these findings indicate that chronic bacterial infections play an important role in the development of wasting as well as immunologic deficiency in neonatally thymectomized rats. It remains to be shown whether infection, bacterial or viral, is of importance in the development of wasting and immunologic deficiency in species other than rats. It is also indicated to gauge the effect on neonatal thymectomy in animals reared in germ-free environments.

Recently, it has been assumed that the cortisol-induced wasting of mice, clinically similar to that of runt disease or post-thymectomy wasting is the result of widespread metabolic dysfunction rather than infection (7). However, it has been shown that antibiotics significantly prolonged the life and reduced the wasting of young rats treated daily with massive doses of cortisone(8). Likewise, the marked weight loss and increased mortality following acute vit. A deficiency in rats are favorably influenced by antibiotic treatment(9).

It is interesting to note also that wasted thymectomized rats, like other thymectomized rats or sham-operated controls, are capable of developing plasma cells and synthesizing immunoglobulins(10). It is postulated that the immunological deficiency of neonatally thymectomized animals is at least partly an expression of immunological preoccupation with infection. On the other hand, the marked susceptibility of thymectomized rats to sepsis indicates some form of participation of the thymus in "natural" or acquired resistance. Whether this participation is in the form of a direct contribution to the total pool of lymphocytes or in the form of a humoral factor(12,13), or both, remains to be clarified.

TABLE III. Anti-Horse Serum Precipitin Reaction in Neonatally Operated Rats (Ouchterlony Double Diffusion).

| Treatment and operation | No. rats | Negative or doubtful reaction, % | Positive reaction, % |
|---|---|---|---|
| *Terramycin group* | | | |
| Thymectomy | 21 | 52.4 | 47.6 |
| Controls (non-thymectomized rats) | 49 | 8.2 | 91.8 |
| *Untreated* | | | |
| Thymectomy | 15 | 80.0 | 20.0 |
| Controls (non-thymectomized rats) | 14 | 28.6 | 71.4 |

*Summary.* The incidence of intercurrent bacterial infections and wasting was considerably higher among neonatally thymectomized rats than in splenectomized or sham-operated controls. Administration of oxytetracycline or implantation of thymus autografts immediately after thymectomy markedly reduced the incidence of bacterial infection and wasting in thymectomized rats. Oxytetracycline-treated thymectomized and con-

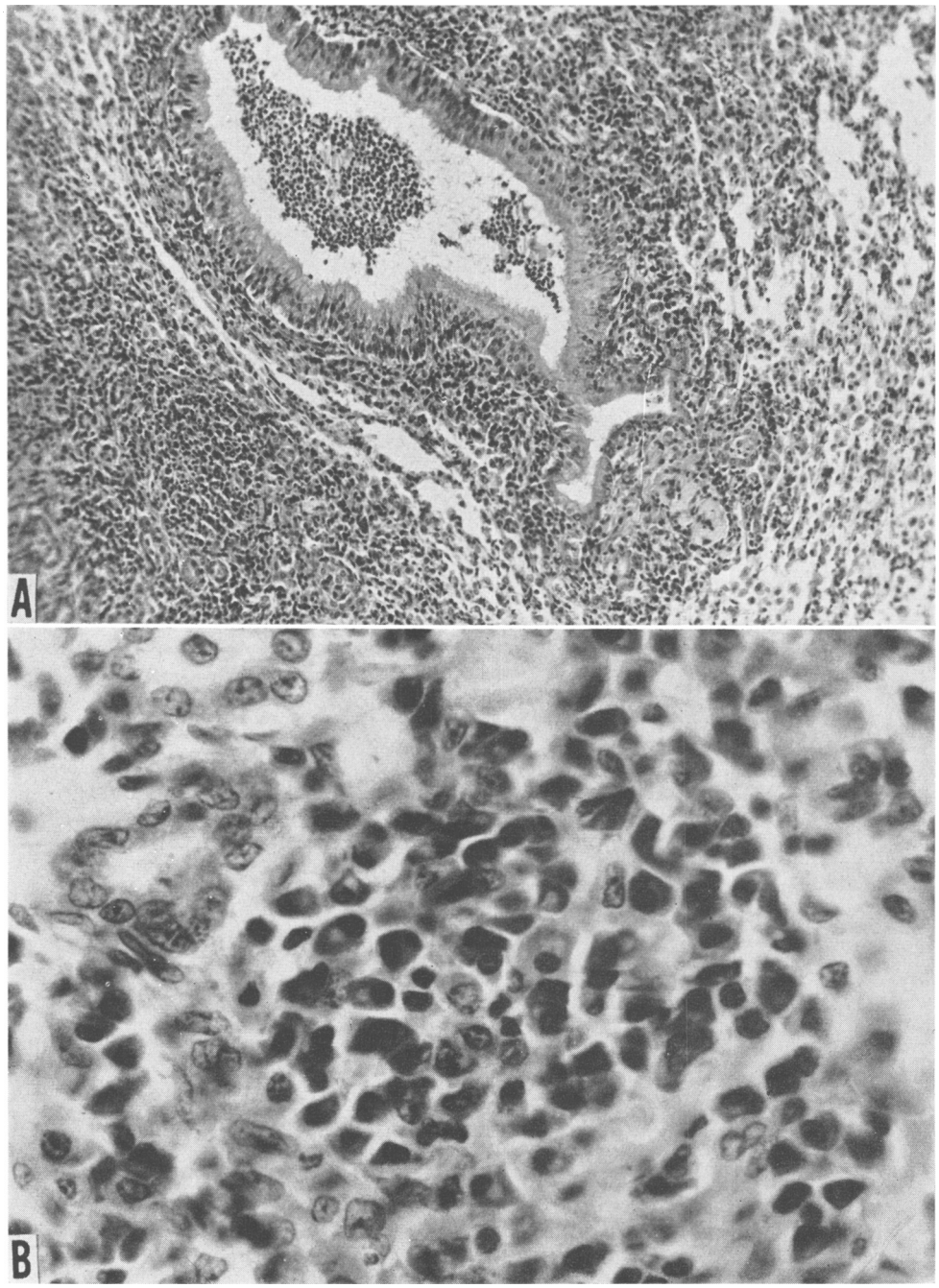


FIG. 3. A field of pneumonia in wasted neonatally thymectomized rat. Top (A): H & E × 108. Bottom (B): H & E × 270. Note predominance of plasma cells.

trol animals showed a considerably more effective antibody response to horse serum than untreated controls. Bacterial infection, usually in the form of abscesses in the region of the ablated thymus or broncho-pulmonary inflammation, was noted in all wasted animals. A predominantly plasmacytic inflammatory reaction and elevation of serum gamma globulin level were observed in most wasted neonatally thymectomized rats in spite of their lymphopenia and splenic atrophy. The findings suggest that bacterial infections, presumably acquired early in life, contribute to the subsequent development of immunologic deficiency and wasting in neonatally thymectomized rats.

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## *In vitro* Cytotoxicity in Delayed-Type Hypersensitivity and Immunologic Tolerance.* (29384)

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Since the early observations of Holst(1) and the classic work of Rich and Lewis(2,3) with tuberculin, an impressive body of evidence has accumulated in support of the phenomenon of *in vitro* toxicity of an antigen for explanted cells from a donor exhibiting delayed-type hypersensitivity. These so-called cytolytic studies have been extended to a number of microbial delayed-type reactivities including streptococcal M substance (4), histoplasmin(5), mumps antigen(6), and brucellergin(7). The only investigation using a haptenic system in which delayed skin reactivity may be selectively engendered was that of Everett *et al* who failed to note any toxic effect on epithelial cells with 2,4 dinitrochlorobenzene or Rhus oleoresin(8). There have been other such negative results with microbial antigens where cells of epithelial origin were used(9,10). All of these cytolytic or cytotoxic studies have served as the sole *in vitro* measure of delayed-type reactivity.

The experiments to be described were designed to observe whether leukocytes from

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