a round cell, often practically indistinguishable from the basophilic round cell, resulting from transformation of the sarcomatous fibroblast.

In view of these findings, the discussion, which cell type in the sarcoma culture, the fibroblasts or the "macrophages" carries the malignant attributes, appears to be futile. The pure Rous sarcoma consisting of one cell type; a mesenchyme cell, appearing at times as a spindle cell, at others as a basophilic round cell is the actual carrier of the Rous sarcoma agent. The changes induced by the sarcoma agent in the spindle cells and basophilic round cells will be described in another communication.

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## Age Variations in Resistance of Albino Rat to Diphtheria Bacilli and to Diphtheria Toxin.

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As reviewed in a previous paper on experimental diphtheria in the albino rat[1] this animal possesses a high but not complete resistance to diphtheria toxin and to living diphtheria bacilli. Amounts of toxin exceeding by 2000 times the fatal dose for guinea pigs (calculated on body-weight) are required to kill albino rats by subcutaneous injection. Again, when large numbers of living bacilli are injected into these animals by the same route only slight transitory lesions are observed. Attempts to explain the mechanism of this natural resistance have been made by several authors. Thus Goodman,[2] Pettit,[3] Coca, Russel and Baughman,[4] Sbarsky[5] have thought that the rat's body-cells are incapable of fixing diphtheria toxin; Ledingham,[6] on the other hand, has expressed his belief that the reticulo-endothelial system of the rat is particularly capable of aiding in the absorption of localized abscesses in the subcutis

1 Seligmann, E., and Jungeblut, C. W., *J. Immunol.*, 1941, **40**, 119.

2 Goodman, H. M., *J. Infect. Dis.*, 1907, **4**, 509.

3 Pettit, A., *Ann. Inst. Pasteur*, 1914, **28**, 663.

4 Coca, A. F., Russel, E. F., and Baughman, W. H., *J. Immunol.*, 1921, **6**, 387.

5 Sbarsky, *Biochem. Z.*, 1926, **169**, 113.

6 Ledingham, J. G. C., *J. State Med.*, 1926, **34**, 2.

and to prevent a dissemination of the bacilli. However, that at least a temporary generalization of the bacilli does occur was suggested by our experiments in which it proved possible to recover diphtheria bacilli from the spleen of infected animals.[1]

The assumed peculiarities of the body-cells in that respect may be connected either with some general phenomena of metabolism or with the specialized function of certain organs that are supposed to be involved in the mechanism of diphtheria intoxication. Since the adrenal glands, for a long time, have been known to be heavily affected in experimental diphtheria, attempts have been made to clarify the significance of this organ for the resistance of rats to toxin. It was found, for instance, that adrenalectomized rats succumbed to smaller doses of diphtheria toxin than controls which had been similarly operated on but without removal of the adrenals (Lewis,[7] Belding and Wyman[8]).

The lesions in the suprarenal glands in diphtheria intoxication of guinea pigs are known to be accompanied by a decrease of the vitamin-C contents of this organ (Harde,[9] Torrance[10]). That this vitamin may play a definite part in the interaction between toxin and tissue was indicated by the investigations of Jungeblut and Zwemer,[11] Greenwald and Harde,[12] and lately again by Jungeblut,[13] who demonstrated that neutral salts of ascorbic acid detoxified diphtheria toxin *in vitro*. It is generally recognized that C-avitaminosis cannot be produced experimentally in the albino rat because this animal synthesizes this vitamin by itself; however, a diet deficient in vitamin A or B produces a definite avitaminotic state. In experiments by Werkman, Baldwin and Nelson[14] such avitaminosis was accompanied by a marked decrease in resistance to diphtheria toxin. An increased resistance, on the other hand, was demonstrated by Meyer[15] in the course of a high-protein diet. Since a similar increase could be observed following inanition the latter was

7 Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 506.

8 Belding, D. L., and Wyman, L. C., *Am. J. Physiol.*, 1926, **78**, 50.

9 Harde, E., *C. R. Acad. Sciences*, 1934, **199**, 618.

10 Torrance, *J. Biol. Chem.*, 1940, **32**, 575.

11 Jungeblut, C. W., and Zwemer, R. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1229.

12 Greenwald, C. K., and Harde, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1157.

13 Jungeblut, C. W., *J. Infect. Dis.*, in press.

14 Werkman, C. H., Baldwin, F. M., and Nelson, V. E., *J. Infect. Dis.*, 1924, **35**, 549.

15 Meyer, A. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 404.

therefore regarded as operating essentially as equivalent to a high-protein diet.

It is generally acknowledged that diet and vitamin-storage are intimately connected with the rate of growth of young animals. Thus the suckling period represents a course of high-protein diet; at the same time the vitamin-C content of the adrenals has been found appreciably lower in young rats than in adult animals.[16] Moreover, the lack of differentiation in the cells of the spleen in very young rats[17] may be of additional significance for the mechanism of natural defense. It has, therefore, seemed justified to investigate the degree of natural resistance to diphtheria bacilli and to toxin in very young rats, all the more so since an increased susceptibility of young animals to diphtheria toxin has already been reported for guinea pigs, dogs, birds, and white mice.[18]

*Experiment 1.* Bacillary infection: Rats, 10 days of age (average weight 12 g), and rats just weaned, 25 days old, (average weight from 40 to 60 g) were infected by subcutaneous injection with 1/10 of the growth of a Lœffler slant (3.8″ x 0.3″ in size) emulsified in saline. When these rats were killed at intervals of 2, 4, 7, and 9 days after infection these animals presented lesions similar to those typical for adult rats. They consisted of a small encapsulated abscess at the site of the injection which contained pus as well as free and phagocyted diphtheria bacilli; at times cultivable diphtheria bacilli were found in the spleen. Rats that had not been killed survived in apparently good health. Thus no difference could be detected in the natural resistance of albino rats to bacillary infection whether the animals were of suckling age, just weaned, or had reached maturity.

*Experiment 2.* Intoxication: The effect of diphtheria toxin was studied in 3 groups of rats: 20 animals of suckling age (12 days old and weighing from 12 to 30 g), 12 rats which had just been weaned (25 days old and weighing from 33 to 48 g), and 15 adult rats (125 to 265 g in weight). All animals received varying doses of toxin by the subcutaneous route. The results are given in Table I.

The table reveals an increased susceptibility of young rats to diphtheric intoxication. Thus, suckling animals died with a minimal dose of 3 mld per g of body-weight, weaned animals of somewhat higher age and weight required about 5 mld, while adult

16 Clark, A. R., personal communication.

17 Perla, D., in Perla, D., and Marmorstone, J., *Natural Resistance and Clinical Medicine*, Boston, Little, Brown & Co., 1941, p. 356.

18 Ssacharoff, G. P., *Ergebn. d. allg. Pathol. und pathol. Anat.*, 1928, **22**, 201.

TABLE I.
Susceptibility of Albino Rats of Different Age and Weight to Diphtheria Toxin.

| Avg age | Wt, g | Toxin in mld per g | No. of animals | Survived | Succumbed | Avg incubation period, days |
|---|---|---|---|---|---|---|
| 12 days | 12-30 | < 1 | 6 | 6 | — | — |
| | | 1 | 2 | 2 | — | — |
| | | 2 | 3 | 3 | — | — |
| | | 3 | 3 | — | 3 | 5.7 |
| | | 4 | 2 | — | 2 | 5.0 |
| | | 5 | 1 | — | 1 | 4.0 |
| | | 6 | 3 | — | 3 | 2.3 |
| 25 " | 33-48 | 3 | 3 | 3 | — | — |
| | | 4 | 3 | 2 | 1 | 7.0 |
| | | 5 | 3 | 1 | 2 | 7.0 |
| | | 6 | 3 | — | 3 | 8.3 |
| Adult rats | 125-265 | 5 | 2 | 2 | — | — |
| | | 6 | 2 | 2 | — | — |
| | | 7 | 3 | 1 | 2 | 8.0 |
| | | 8 | 8 | — | 8 | 5.6 |

rats succumbed only to doses of at least 7 mld. The dead rats showed a hemorrhagic edema of the lungs, frequently pleural effusion and sometimes congestion of the adrenal glands.

These data indicate that there may be a difference in age-resistance to diphtheria toxin but not to bacillary infection. This fact is readily explained since even the most susceptible suckling animal succumbs only to comparatively large doses of toxin, *i. e.*, 3 mld per g body weight. It is not to be assumed that such an amount of toxin ever accumulates in the animal's body during the course of experimental infection with diphtheria bacilli.

*Conclusion.* Young albino rats are more susceptible to diphtheria toxin than are adult animals. No difference seems to exist between young and old rats regarding their resistance to bacillary infection.