

produced by a natural pelleted diet(2).

Conclusion. The thiamine requirement of the guinea pig was estimated with a purified diet prepared with two different salt mixtures. In the first experiment an apparently high requirement for thiamine was traceable to a rapid destruction of the vitamin with the diet stored at $+5^{\circ}\text{C}$. With a different salt mixture, designed to minimize the storage changes, a requirement of 2 mg of thiamine/kg of diet was found.

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1. Reid, M. E., Proc. Soc. Exp. Biol. and Med., 1954, v85, 547.
2. Reid, M. E., Briggs, G. M., J. Nutrition, 1953, v51, 341.
3. Briggs, G. M., Spivey, M. R., Keresztesy, J. C., Silverman, M., Proc. Soc. Exp. Biol. & Med., 1952, v81, 113.
4. Fox, M. R. Spivey, Briggs, G. M., J. Nutrition, 1960, v72, 243.
5. Waibel, P. E., Bird, H. R., Baumann, C. A., *ibid.*, 1954, v52, 273.
6. Fox, M. R. pivey, Mickelsen, O., *ibid.*, 1959, v67, 123.
7. National Research Council, Nat. Acad. Sci., Washington, D. C., 1962, Publication 990.

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Effect of Thymectomy on Rous Virus Tumor Growth Induced in Chickens. (32355)

R. RADZICHOVSKAJA (Introduced by C. M. Southam)

Institute of Experimental and Clinical Oncology of Medical Sciences, Moscow, USSR

Neonatal thymectomy has a pronounced inhibiting or stimulating effect on the tumor growth induced by various oncogenic viruses (2,3,6,7,9-15,21). Our objective was to investigate the role of the thymus on the induction and growth rate of Rous virus sarcoma in chickens.

Material and methods. Chickens were thymectomized during the first 72 hours of life. Thymectomy was done by the technique described by Peterson *et al*(15). As controls we used intact or sham-operated chickens of the same age as the thymectomized ones. The experimental and control birds were simultaneously inoculated with the Rous sarcome virus (strain Carr-Zilber) at the age of 10-12 days. As a source of Rous sarcoma virus we used a cell-free tumor extract, which was stored in the frozen state (at -70°C) for 6 months. The virus titer, which was estimated by means of virus injection into 1-day chicks, was 1×10^6 LD₅₀/ml. All experiments were made with the same pool of tumor extract. Each thymectomized and control chicken was inoculated with the virus in a dilution of 10^{-5} or 10^{-6} intracutaneously in both wings. All chickens were observed

for 30 days after virus injection. During this period we examined the chickens for tumors 3 times each week and autopsied every dead chicken. All birds which remained alive to the end of the experiment were sacrificed and autopsied.

Results. The results of our experiments are summarized in Table I and Fig. 1 and 2.

The average time of tumor appearance in the thymectomized chickens was 10-12 days. In most control birds the tumors appeared 3-4 days later. The 10^{-5} dilution of virus caused tumors in each of the 42 thymectomized chicks (100%) and in 44 of the 52 controls (84.6%). The 10^{-6} dilution of virus caused tumors in 20 of 42 thymectomized chicks (47.6%) and in 13 of the 52 control birds (25.0%). Among the thymectomized chickens, in addition to the increased frequency of tumors, the tumors also grew more rapidly and killed sooner. By the end of the experiment there had been 36 deaths due to tumors among the 42 thymectomized chickens (85.7%) and in only 10 of 44 control chickens (22.8%). The probability value (P) by Chi² analysis was $P < 0.001$.

The primary intracutaneous tumors in the

TABLE I. Tumor Growth in Thymectomized and Control Chickens Inoculated with Rous Sarcoma Virus.

Virus dilutions	Thymectomized chickens			Control chickens		
	Primary induced tumors*	Death due to tumors†	Metastases‡	Primary induced tumors	Death due to tumors	Metastases
10^{-5}	42/42 (100%)	36/42 (85.7%)	18/42 (42.8%)	44/52 (84.6%)	10/44 (22.8%)	5/44 (11.1%)
10^{-6}	20/42 (47.6%)			13/52 (25.0%)		

* No. with tumors/No. inoculated chickens.

† No. deaths due to tumors/No. chickens with tumors.

‡ No. deaths due to metastases/No. chickens with tumors.

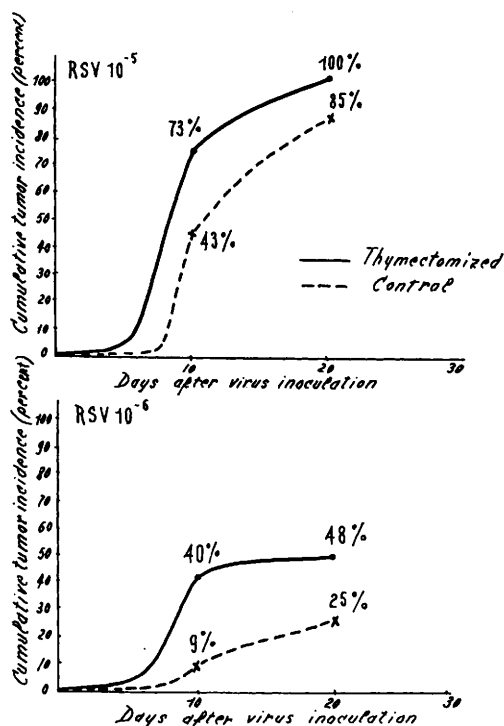


FIG. 1. Tumor incidence in thymectomized and control chicks inoculated with Rous Sarcoma Virus (RSV).

thymectomized chickens metastasized to internal organs more often than did the primary tumors in control chickens. The diagnosis of metastases was based on gross criteria. Microscopic examination was made only in some doubtful cases. The incidence of metastases among birds injected with 10^{-5} RSV was 42.8% in thymectomized and 11.1% in control birds ($P < 0.001$). The location of metastases was approximately the same in both groups. They were most frequent in liver and lungs and sometimes were found in the

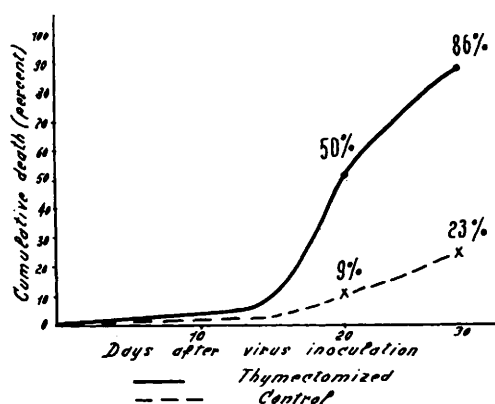


FIG. 2. Death due to tumors of thymectomized and control chicks.

heart.

Discussion. The experiments described here showed that neonatal thymectomy has a stimulating effect on the induction and growth rate of the Rous virus sarcoma in chickens. The probable explanation might be as follows:

Several authors(4,5,8,16,17,19,20,23) have presented evidence from transplantation studies which suggested that the virus-induced tumor cells contained a "new" antigen (or antigens) and that animals react against this tumor antigen with an immune defense reaction of a homograft type. In the immunologically disturbed chickens (following neonatal thymectomy) this defense mechanism was suppressed. Therefore the transformed neoplastic cells clones grow more progressively in the thymectomized chickens than in the control ones. In a previous paper(18) it was shown that bursectomy had no significant effect on Rous virus sarcoma induction and tumor growth rate in chickens. The

Bursa of Fabricius is known to play a principal role in regulating formation of humoral antibodies, and the thymus is known to play a principal role in cellular (lymphocyte) immunity in birds(1,22). Our results further support the concept that cellular immunity plays an important part in the resistance of the host against tumor formation and that humoral antibodies are not importantly involved in this resistance.

Summary. Chickens were thymectomized during the first 72 hours of life. Controls were intact or sham-operated chickens of the same age. All chickens were simultaneously inoculated with Rous sarcoma virus at the age of 10-12 days. Thymectomy had a stimulating effect on induction of the Rous sarcomas and tumor growth rate.

1. Cooper, M., Raymond, D., Peterson, A., South, M., Good, R., J. Exp. Med., 1966, v123, 75.
2. Defendi, V., Roosa, R., Cancer Res., 1965, v25, 300.
3. Gross, L., Proc. Soc. Exp. Biol. & Med., 1959, v100, 325.
4. Habel, K., ibid., 1961, v106, 722.
5. Habel, K., Eddy, B., ibid., 1963, v113, 1.
6. Yohn, D. S., Funk, C. A., Grace, J. T., Proc.

Am. Assn. Cancer Res., 1965, v6, 70.

7. Kirschstein, R., Rabson, A., Peters, E., Proc. Soc. Exp. Biol. & Med., 1964, v117, 198.
8. Klein, G., Sjöggren, H., Klein, E., Cancer Res., 1962, v22, 955.
9. Law, L., Nature, 1965, v205, 672.
10. ———, Cancer Res., 1966, v26, 1121.
11. Law, L., Ting, R., Proc. Soc. Exp. Biol. & Med., 1965, v119, 823.
12. Malmgren, R., Rabson, A., Garney, P., J. Nat. Cancer Inst., 1964, v33, 101.
13. Martinez, C., Nature, 1964, v203, 4950, 1188.
14. Miller, I., ibid., 1959, v183, 1069.
15. Peterson, R., Burmester, B., Fredrickson, T., Purchase, G., Good, R., J. Nat. Cancer Inst., 1964, v32, 1343.
16. Prigogina, E., Stavrovskaja, A., Nature, 1964, v201, 934.
17. Radzichovskaja, R., ibid., 1964, v204, 392.
18. ———, ibid., 1964, v213, 1259.
19. Sjöggren, H., Virology, 1961, v15, 214.
20. Sjöggren, H., Jonsson, N., Exp. Cell Res., 1963, v32, 618.
21. Stepina, V., Mazurenko, N., Neoplasma, 1966, v13, 265.
22. Szenberg, A., Warner, N., Nature, 1962, v194, 146.
23. Trentin, I., Bryan, E., Proc. Am. Assn. Cancer Res., 1964, v5, 64.

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Effect of Antimicrobial Drug Combinations on Enzymic Lysis of *Staphylococcus aureus*. (32356)

GEORGE H. WARREN AND JANE GRAY

Research Division, Wyeth Laboratories, Inc., Radnor, Pa.

Although there have been numerous investigations of the antibacterial activities of antibiotic combinations and certain pairs of drugs have often been reported as synergistic or antagonistic, many of the reports are conflicting(1-6). Frequently the results of published studies reveal that the antagonistic or synergistic effect depends to a great extent upon the specific species or strain of bacteria employed and the relative proportion of the agents to which it has been exposed(1,5).

Previous studies from this laboratory(7-9) have demonstrated that the semisynthetic penicillin nafcillin has a profound effect on the integrity of the *Staphylococcus aureus*

cell wall; in sublethal concentration, nafcillin renders the cell highly susceptible to lysis by lysozyme and trypsin.

An attempt has now been made to use this lytic system as a model for determining the activities of antibiotic combinations. In the present work, several other antibiotics were compared with nafcillin for the effect on the enzymic lysis of *S. aureus* CHP, and in addition their effect when combined with nafcillin was determined. The drugs presently investigated in this manner were tetracycline, chloramphenicol, novobiocin, erythromycin, actinomycin D, ampicillin, cephalothin and cloxacillin. Our observations that