experiments. After the pulmonary artery and its branches have been freed and the right branch prepared as above, the main artery is occluded centrally, and the heart is fibrillated by means of an inductorium. A cannula, like that in Fig. 1, is quickly inserted into the artery and tied in place with the circulation at a standstill. The heart is then defibrillated with countershock, according to the method of Wiggers(5), and an active circulation is reestablished. The rotameter assembly unit is finally attached to the cannula. Success with this approach is limited by the time necessary for cannulation; for if fibrillation persists longer than 1 to 2 minutes, countershock alone is usually ineffective in terminating the fibrillation. Cardiac massage must then be combined with countershock to restore the heartbeat, and the reestablished circulation may not be adequate. However, if cannulation is completed within 1 to 1½ minutes, revival is usually easy and the beat is sufficient to maintain an adequate output.

The pulmonary artery has been cannulated successfully in 9 of 12 dogs, and flows recorded by the rotameter range from 40 to 100 cc per kilo.

Acknowledgment is made of the technical assistance given in these experiments by Edward M. Khouri, and F. H. Longino.

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## Antagonistic Action of Certain Neurotropic Viruses Toward a Lymphoid Tumor in Chickens with Resulting Immunity.* (17651)

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Moore(1) reviewed the literature on the growth of viruses in tumors and reported the tumor-necrotizing action of the Russian Far East encephalitis virus on mouse sarcoma 180(2). In the report that follows, results are presented of experiments carried out with the Russian Spring-Summer encephalitis virus (RSS) in domestic chickens infected with a transplantable malignant lymphoid tumor (3,4). A number of other viral and rickettsial agents also were tested, results of which are recorded briefly.

*Experimental tumor.* The RPL-12 lymphoid tumor(5) originally described by Olson (6) was received through the kindness of Drs. B. R. Burmester, G. E. Cottral and B. Winton, Regional Poultry Research Laboratory, U. S. Department of Agriculture, East Lansing, Mich. Because on storage there is considerable loss of infectivity, freshly harvested tumors were used in each experiment. Tumors were removed in early stage of growth and homogenized with saline solution in a TenBroeck grinder(7). Unless otherwise stated, the infecting dose used was 0.25 ml of 1:1,000 suspension of tumor tissue injected into the pectoral muscle.

*Viral and rickettsial inocula.* The following viruses were used: mumps (Enders strain), swine influenza (Shope strain No. 15) in form of infected allantoic fluid from chick embryos; canine distemper (Lederle strain) as infected chorioallantoic membrane; Columbia-SK, Bwamba (M483 strain(8)), Semliki Forest (9), Ilheus encephalitis(10), Theiler's GD VII mouse encephalomyelitis(11), eastern

* Our interest in virus-tumor antagonism was stimulated by personal communications from Dr. C. P. Rhoads, Director, Memorial Hospital, and the Sloan-Kettering Institute for Cancer Research, prior to the publication of Dr. Moore's results.

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TABLE I.
Relationship of Variations in Age of Host and Tumor to Response Following Injection of RSS Virus.

| Age of tumor,* days | Age of bird, days | RSS treated† | | Untreated controls | |
|---|---|---|---|---|---|
| | | Survival ratio | % survival | Survival ratio | % survival |
| 2 | 1 | 14/18 | 78 | 3/20 | 15 |
| 6-9 | 1-2 | 2/38 | 5 | 1/32 | 3 |
| 2-4 | 7 | 36/48 | 75 | 0/33 | 0 |
| 5-6 | 7 | 26/28 | 93 | 6/20 | 30 |
| 7-9 | 7 | 9/41 | 22 | 1/51 | 2 |
| 2-4 | 14 | 99/112 | 88 | 6/95 | 6 |
| 7-9 | 14 | 39/69 | 57 | 15/68 | 22 |
| 2-3 | 21 | 27/28 | 97 | 4/29 | 14 |
| 9 | 28 | 15/26 | 58 | 5/24 | 21 |

* The number of days elapsing between tumor inoculation and injection of RSS virus is termed "Age of Tumor."

† Virus inoculum consisted of 0.5 ml of 1:100 dilution of RSS-infected mouse brain.

equine encephalomyelitis as infected mouse brain suspensions; western equine encephalomyelitis as infected whole chick embryo; the Pitman-Moore No. 980 strain of rabies virus as a suspension of infected rabbit brain, the Flury strain(12,13) and Novisad strain(14) of rabies virus as saline suspensions of whole chick embryo. The RSS(15), West Nile (M-956 strain(16)), Japanese B encephalitis (Nakayama strain(17)), St. Louis encephalitis (Brown strain) and louping-ill viruses were used in form of infected mouse brain tissue suspensions. The $LD_{50}$ titers in intracerebrally injected 28-day-old mice were: RSS $10^{-5}$ to $10^{-6}$; West Nile $10^{-8}$ to $10^{-9}$; Japanese B encephalitis $10^{-5}$; St. Louis encephalitis $10^{-4.5}$, and louping-ill $10^{-5.5}$. Rocky Mountain spotted fever (Montana R strain), North Queensland tick typhus (PHS strain), rickettsial pox (McCauley strain), boutonneuse fever (Moroccan strain No. 1), South African tick bite fever (Malish No. 7 strain), epidemic typhus (Breinl strain), murine typhus (Wilmington strain), *Lymphogranuloma venereum* (Nigg strain), feline pneumonitis (Baker strain), human pneumonitis (SF strain), and bovine encephalitis (McNutt strain) were used as infected yolk-sac tissue suspensions.

*Chickens.* All of the experiments were done with New Hampshire Red chicks. Since all lots of chicks tested were susceptible to the tumor, no attempt was made to obtain all birds from the same flock.

*Relationship of variation in age of host and of tumor to response following RSS-virus inoculation.* Since the results of a preliminary experiment indicated that RSS virus had some oncolytic action, and that the age of the bird may have some bearing on the outcome, additional birds ranging in age from 1 to 28 days were inoculated with tumor and 2 to 9 days later were injected in the tumor or tumor-inoculation site with 0.4 to 0.5 ml of 1:100 RSS-infected mouse brain suspension. It was observed that tumors continue to grow for a few days after infection with RSS virus and although frequently no difference in size can be seen for 6 to 7 days, the virus-treated

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TABLE II.
Extra-Tumor Inoculation of RSS Virus in 2-week-old Chicks.

| Age of tumor, days | RSS-infected mouse brain* | | 6th or 8th passage RSS in tumor† | |
|---|---|---|---|---|
| | Treated | Untreated | Treated | Untreated |
| | Survival ratio | | Survival ratio | |
| 1 | 4/10 | 1/9 | | |
| 2 | 7/8 | 1/9 | 15/20 | 1/19 |
| 3 | 7/10 | 1/9 | 10/10 | 1/10 |
| 4 | 6/10 | 1/9 | 20/20 | 1/19 |

* .5 ml of 1:100 RSS-infected mouse brain (mouse $LD_{50}$ titer $10^{-5.5}$).
† .5 ml of 1:100 RSS-infected tumor tissue (mouse $LD_{50}$ titer $10^{-5.5}$).

tumors tend to be less firm. In untreated chicks up to 2 weeks of age the tumors grow rapidly and most of the birds die within 8 days after the tumor becomes palpable, whereas tumors in older birds attain a greater relative size and are associated with a longer survival time. Many of the chicks treated 2 days after tumor inoculation did not develop palpable tumors, whereas most of those treated 4 days after tumor-inoculation did, but the tumors remained small and regressed completely within 5 to 10 days. Tumors occasionally failed to develop in chicks which were not treated. From the data presented in Table I it may be noted that regardless of the age of the birds, when RSS virus was introduced into the tumor site 2 to 4 days after inoculation a high percentage of the treated birds survived, but the higher survival rates among the untreated controls at the 2 to 4 week age levels suggest that an age-resistance factor may have some influence at this time.

*Effect of mixed RSS virus and tumor inoculum.* Since the survival ratios were much greater when RSS virus was introduced 2 to 4 days after tumor inoculation, it was of interest to determine what the effect would be when the inoculum consisted of a mixture of tumor suspension and RSS virus. One group of 9 chicks received 0.25 ml of a mixture containing 1:100 tumor suspension (10 times the usual infecting dose) and 1:100 dilution of RSS-infected mouse brain, which was held at 4° C for 1 hour prior to injection. All of the birds survived but on the 8th day 2 of them had small tumors which, however, regressed completely within the next 7 days. The second group of chicks was given a similar mixture except that the RSS infected mouse brain was diluted 1:10,000. Nine of the 10 birds survived, but 3 of the survivors showed palpable tumors which later regressed. Of the 15 control birds none survived.

*Effect of mouse brain tissue on tumor growth.* Two-week-old chicks were treated 3 days after tumor inoculation with 0.5 ml of 1:100 suspension of normal mouse brain tissue given into the tumor-injection site. Of the 29 so treated 7 survived (24%) while of the 19 tumor-bearing untreated control chicks 6 survived (31%). Further evidence that mouse brain tissue had no oncolytic action was provided by the failure of treatment with such tissue infected with other viruses. In all, 98 chicks were so treated and of these only 9 (9%) survived while of 95 in the untreated control groups 7 (7%) survived.

*Effect of extra-tumor injection of virus.* While the data in Table II show that inoculation with RSS-infected mouse brain suspensions into breast opposite to that of tumor inoculation saved a significant number of birds, it should be stated that most of the tumors became large before regressing, but treated chicks that died with tumors had a longer survival period than the untreated controls. Tumor-regression was likewise obtained when the inoculum comprised 6th or 8th passage RSS virus in the RPL-12 tumor†

† The passages of RSS virus in RPL-12 tumors were made in chicks with palpable tumors by injecting into the side of the breast opposite the tumor 0.5 ml of 1:100 suspension of virus-infected tumor from the previous passage. Three or 4 days after the birds were infected with virus, the tumors were harvested, made to a 20% suspension in distilled water in a Waring blendor and stored at —30°C.

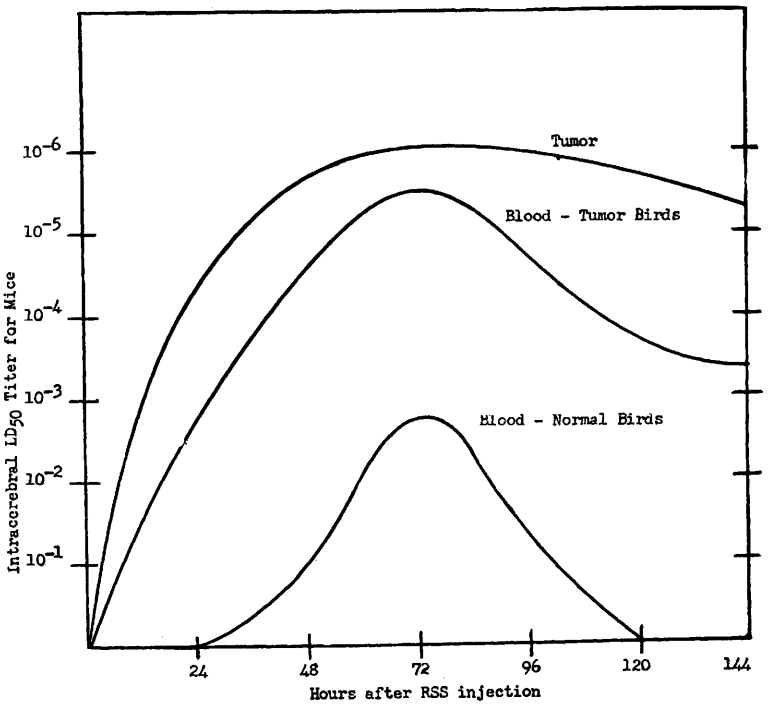


FIG. 1.
Concentration of RSS virus in blood of normal, and blood and tumor of tumor-bearing chicks at intervals following infection.

introduced into the side opposite to the tumor inoculation. A comparison of the data shown in Table II with those in Table I indicates that RSS virus obtained from tumors is as effective when given at a distance from the tumor as is RSS virus-infected mouse brain given into the tumor-inoculation site. That the difference is qualitative rather than quantitative is indicated by the fact that the mouse $LD_{50}$ titers were the same for each type of inoculum.

*RSS virus titer in the tumor.* A group of 20 chicks with palpable tumors in the left pectoralis was given in the opposite breast 0.5 ml of 1:100 dilution of RSS-infected mouse brain suspension. At each successive 24-hour interval for 6 days, 3 chicks of the tumor-bearing, virus-treated group were bled and an equal amount of blood from each was pooled. At the same time the tumors were removed and pooled. A suspension of each pool was titrated intracerebrally in mice to determine the $LD_{50}$ titer of the RSS virus present in the blood and in the tumor tissue. Blood from a group of 20 nontumor-bearing birds inoculated with RSS virus was treated in similar manner. From the results shown in Fig. 1, it is apparent that the virus concentration was the highest, and persisted the longest, in the tumor tissue.

*Experiments with West Nile, St. Louis, Japanese B encephalitis and louping-ill viruses.* Most of the chicks survived inoculation of tumor in one side of the breast when they were treated in the same side 3 to 4 days later with any one of the above 4 viruses (Table III). While the relative oncolytic effectiveness of these agents remains to be determined, the impression gained was that the tumors exhibited a delayed and somewhat variable response to the West Nile virus and that the response to Japanese B encephalitis virus was also delayed. The reaction to St. Louis encephalitis and louping-ill viruses was similar to the best that was obtained with RSS virus.

*Effect of other viral and rickettsial agents.* Of all the other viral and rickettsial agents tested in a similar manner to that described

TABLE III.
Two-week-old Chicks Treated 3 or 4 Days After Tumor Inoculation with Suspensions of Mouse Brains Infected with Viruses Other Than RSS.

| Virus | Virus dose ml | Virus dose % | Virus-treated Survival ratio | Untreated controls Survival ratio |
|---|---|---|---|---|
| West Nile | .5 | 10 | 13/18 | 0/24 |
| | 1 | 10 | 19/25 | 2/37 |
| | .5 | 1 | 8/10 | 3/10 |
| Japanese B | .5 | 1 | 16/20 | 1/18 |
| | .5 | .1 | 9/10 | 0/7 |
| St. Louis | .5 | 1 | 19/19 | 1/18 |
| | .5 | .1 | 10/10 | 0/7 |
| Louping-ill | .5 | 1 | 17/20 | 2/17 |
| | .5 | .1 | 8/10 | 2/10 |
| | .5 | .01 | 10/10 | 2/10 |

for the above viruses, none showed any consistent oncolytic action.

*Immune status of surviving birds.* Chicks, in which RPL-12 tumors have regressed, usually remain healthy and are immune to challenge with the homologous tumor(18). Of the survivors from the experiments described above, 185 chicks, including representative groups from all successful forms of treatment, were challenged with the homologous tumor 1 to 3 months after complete regression of all tumors. Of these birds, 55 never had presented any palpable tumors. In each experiment, normal uninoculated birds were set aside to serve as controls in the subsequent immunity-challenge tests. Tumor development seemed a better criterion for comparison of immunity than death or survival and on this basis approximately 95% of the normal control birds proved to be susceptible, while among the 185 challenged survivors only 9 (5%) developed tumors (of these 2 died). Of 18 chicks which had survived the RSS virus-tumor mixture inoculations, 4 (22%) developed tumors after the challenge and 1 died with the disease. In 13 of these 18 chicks no tumors were detected by palpation either following the original or after the challenge tumor-inoculation. Of the total 203 chicks which were challenged, 11 died or were sacrificed in a paralyzed condition. Although a similar type of paralysis occurred in at least 3 unchallenged control chicks, the data are inadequate to give an accurate estimate of the expected incidence of paralysis in chicks of this stock. While the work of Burmester, Prickett and Belding(19) suggests that neural lymphomatosis is probably not associated with the RPL-12 tumor agent, Cottral(20) after analysis of data available, formulated the working hypothesis that immunity to a tumor does not signify immunity to the etiologic agent of lymphomatosis since many of the chicks that survived the tumor transplant died of visceral lymphomatosis at a later date.

*Comments.* Two observations are of interest: first, that 5 different viruses which are relatively non-pathogenic for chicks possess the ability to reverse the course of tumor development which usually destroys its host, and second, that recovered birds are resistant to reinoculation with the homologous tumor. Those viruses reported to be effective in this study have certain properties in common. They are associated with systemic infections, have predilection for nervous tissues and have a fairly wide host range although they may or may not give rise to symptoms in susceptible species. Of further interest is that they fall into two groups: the RSS and louping-ill viruses are closely related as evidenced by neutralization and complement-fixation tests, and by cross-immunity tests(21); the other 3 viruses apparently contain a common minor

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antigen(22). Using the known properties, such as host range, mode of transmission, systemic infection and predilection for nervous tissue of these 5 viruses as a possible pattern of characteristics, it would seem that the eastern and western equine encephalomyelitis viruses would answer the requirements but experiments showed that these agents had no oncolytic effect on RPL-12. Any hopes engendered by the data presented in this report must be tempered by the realization that those agents which were found to be effective are impractical for use in public health or veterinary medicine. In addition, it should be emphasized that it is probably true in chicks, as has been shown for mice(23), that any one viral agent is not effective against all types of neoplasia. It is apparent, therefore, that the search for agents which may be employed with safety must be continued along broad lines and it is possible that effective viral agents may be found which have neither the antigenic nor pathogenic characteristics in common with the neurotropic group reported above.

*Conclusions.* 1. RPL-12, a transplantable lymphoid tumor of chickens, can be caused to regress, without any apparent damage to the host, by superimposed inoculations of Russian Spring-Summer, West Nile, Japanese B encephalitis, St. Louis encephalitis or louping-ill viruses.

2. Chickens which have been inoculated with the RPL-12 tumor followed by a superimposed infection with any of the 5 viral agents are rendered immune, as a rule, to challenge by homologous tumor even though the original inoculation with tumor may not have produced a palpable tumor.

3. The infective titer of RSS virus in infected tumor tissue is equal to that of infected mouse brain.

4. RSS virus obtained from infected mouse brain has a high oncolytic action when introduced into the tumor area but is less effective when it is introduced at a distance from the tumor.

5. RSS virus obtained from infected RPL-12 tumor has a high oncolytic action when introduced either in the tumor area or at a distance from the tumor.

The authors wish to express their appreciation to Miss Margaret Batstone for her able technical assistance during the course of these studies.

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## Activity of *p*-Aminobenzaldehyde, 3-Thiosemicarbazone on Vaccinia Virus in the Chick Embryo and in the Mouse. (17652)

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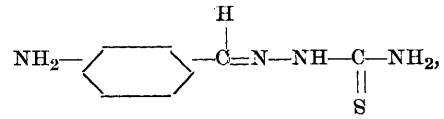
The recent addition of the thiosemicarbazones to the list of therapeutic agents for the treatment of tuberculosis(1-3) led us to investigate some of these compounds for possible activity against other pathogenic microorganisms including some of the viruses. Because it was the most soluble compound in our collection of thiosemicarbazones at the time this work was undertaken, the *p*-aminobenzaldehyde 3-thiosemicarbazone (MC2343),



was selected for first tests against vaccinia virus.

*Vaccinia tests in chick embryos.* In this test,

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