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Bone Tumors in Fowls Injected Intravenously with Causative Agent of Rous Sarcoma.

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It is well known that intravenous injections of the causative agent of filterable fowl sarcoma into fowls never produce widespread sarcomatosis and, in the absence of a primary tumor, seldom cause solitary sarcomas in the visceral organs.[1-7] The agent introduced into the blood stream rapidly disappears from the circulation, probably being taken up by the cells of the reticulo-endothelial system.[8] There is general belief that the sarcoma producing agent injected into the circulation initiates a new growth only at points of vascular injury, *i.e.* where the agent comes into contact with the connective tissue cells. This can take place in the ovary as a sequence of frequent injuries to the vessels occurring in this organ during the process of ovulation.[1] Experimentally the agent can be localized by thermo-cauterisation of the skin,[3] by injections of histamine,[6] by puncture of the vessel,[2] by inducing the development of reaction tissue,[4] etc.

We wish to report in the following our experiments on intravenous injections of the causative agent of Rous sarcoma into chicks, the results of which deviate considerably from previous observations.

In the present study we used White Leghorn chicks of both sexes from the same farm. As a source of the causative agent cultures of filterable fowl sarcoma (strain Rous-Fischer) were employed. This tumor has been maintained in this laboratory for the past 10 years by serial passages *in vitro*. The following design of experiment was adopted: fragments of a Rous cell colony were planted into a Carrel flask containing chicken plasma diluted with Tyrode solution in the proportions of 1:2. After cultivation for 1-3 weeks the flasks were overlaid with Tyrode solution and placed in the incubator for 30 minutes; the supernatant fluid was pipetted off and centrifuged 3 times at 3000 r.p.m. for 15 minutes each time.

Twelve chicks, aged 4 to 10 weeks, in 4 experimental groups were injected with 1 ml of this fluid into the wing vein, 2 or 3 injections being given at weekly intervals to each chick. The fowls succumbed in 21 to 42 days after the initial injection. Necropsy showed new growths in the liver, lungs, heart and kidneys in 9 of the 12 chicks. The tumors in the viscera were always associated with a tumor at the site of the inoculation.

The outstanding feature observed in all chicks was the following: 11 of 12 chicks injected with sarcoma agent displayed most extensive neoplastic bone changes, involving one or several bones (sternum, femur, tibia, tarso-metatarsus, radius, ulna, humerus). Histologically, the tumors were found to be typical osteoid sarcoma destroying the bone and invading the nearby muscles.

The appearance of a general bone neoplasia in a series of animals injected intravenously with Rous agent is noteworthy. This behaviour of Rous agent injected into the blood stream has never been described and in the 10 years during which we have worked with our strain in this laboratory this is the first time that bone tumors appeared either after

1 Rous, P., Murphy, J. B., and Tytler, W. H., *J. A. M. A.*, 1912, **58**, 1751.

2 Pentimalli, F., *Z. f. Krebsforsch*, 1924, **22**, 74.

3 Findlay, G. M., *J. Path. and Bact.*, 1928, **31**, 633.

4 Mackenzie, R. D., and Sturm, E., *J. Exp. Med.*, 1928, **47**, 345.

5 Cramer, W., *Ninth Scientific Report, Imperial Cancer Research Fund*, London, 1930, 21.

6 Doerr, R., Bleyer, L., and Schmidt, G. W., *Z. f. Krebsforsch*, 1932, **36**, 256.

7 Sittenfield, M. J., Johnson, B., and Jobling, J. W., *Am. J. Cancer*, 1932, **16**, 345.

8 Mellanby, E., *J. Path. and Bact.*, 1938, **47**, 47.

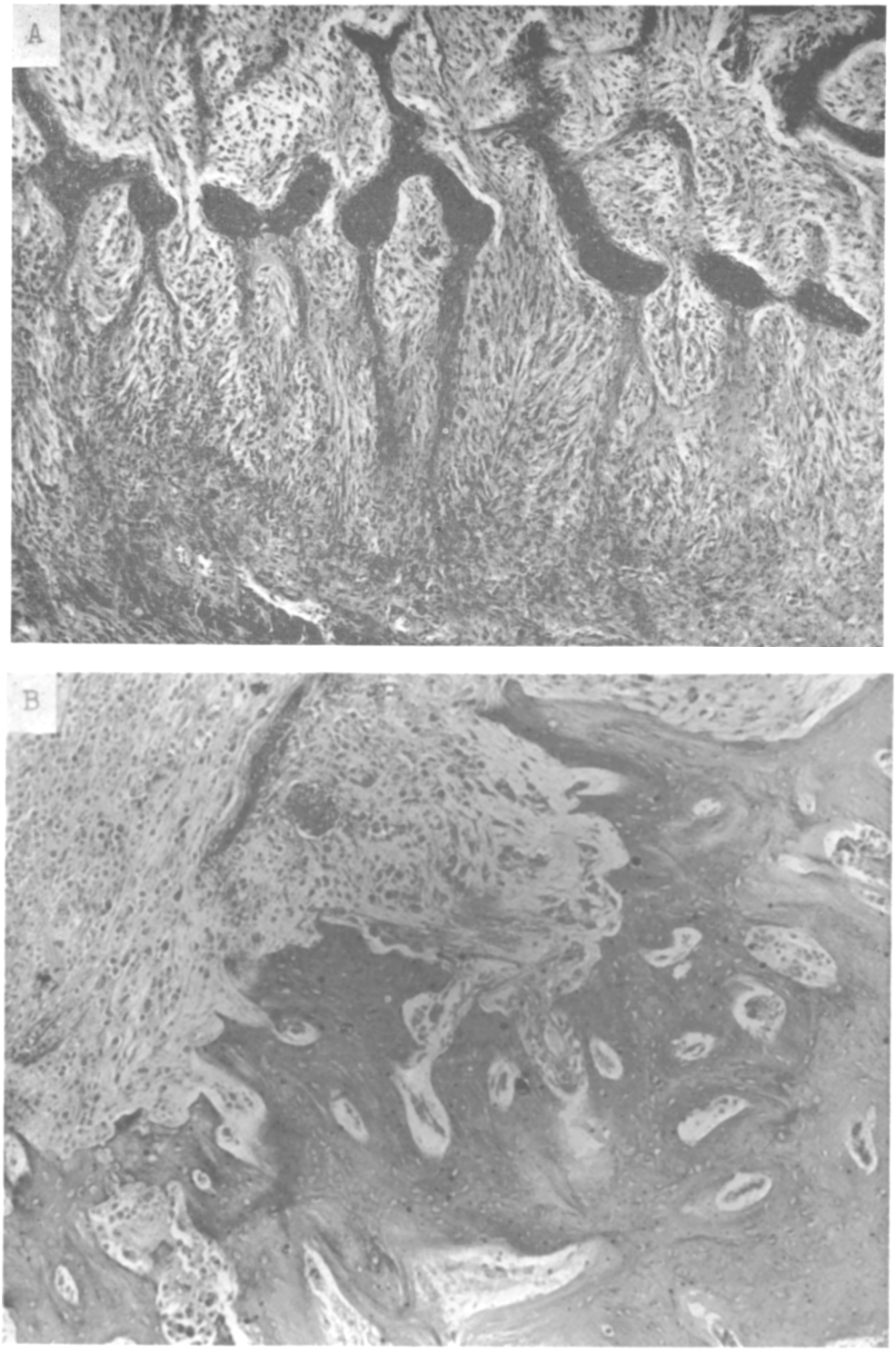

Fig. 1.
a. Chicken N. 3447. Bone tumor. Hematoxylin-Eosin. Mag. X 145.
b. Chicken N. 3449. Bone tumor. Hematoxylin-Eosin. Mag. X 135.

intramuscular or intravenous injections. The cause of this unusual effect of the intravenous inoculation of Rous agent in the experiments reported is entirely obscure. We do not know whether it is to be sought in the particular manner of applying the agent (repeated intravenous injections) or whether we are dealing with a real modification of the agent, which took place spontaneously or resulted from long passage *in vitro*. All these possibilities deserve consideration.

During the preparation of this note a paper of Shrigley, Greene and Duran-Reynolds[9] appeared. The authors observed that the Rous sarcoma agent after remaining in the anterior chamber of the eye of the guinea pig acquired the ability to produce periosteal tumors in chicks. To judge by the description and the photomicrograph of these growths, they are very similar to those here reported. The acquisition of a new tissue affinity was ascribed to modification of the agent caused by its sojourn in an unnatural environment.

Summary. The appearance of an osteoid sarcoma in a series of chickens injected intravenously with the causative agent of Rous sarcoma derived from sarcoma cell cultures, is reported.

[9] Shrigley, E. W., Greene, H. S. W., and Duran-Reynals, F., *Cancer Research*, 1945, **5**, 356.

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Development of Tolerance to Typhoid Bacterial Pyrogen and its Abolition by Reticulo-Endothelial Blockade.*

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Patients who are being given fever therapy by means of intravenous injections of typhoid vaccine exhibit a decreasing reactivity, and must be given larger and larger doses of the vaccine in order to develop similar fevers at successive treatments. After several injections some individuals may require doses as large as 250 ml of typhoid vaccine in a single day.[1] The mechanism of this remarkable tolerance has not been explained. The present report deals with a study of the phenomenon in rabbits.

The rabbits used were males, of mixed breed, weighing 2 to 3 kg. During test periods they were placed in wooden stalls, and held by head boards. Rectal temperatures were taken at 30-minute intervals, through openings in the floors of the stalls. Observations were never continued for more than 7 hours after giving vaccine, to prevent undue fatigue of the animals. The vaccine used contained approximately one billion killed *E. typhosa* per ml.† The dose was 1 ml of a 1:8 dilution in physiologic salt solution. The agent used for blockade of the reticulo-endothelial system was colloidal thorium dioxide (Thorotrast-Heyden Co.); 9 ml was injected intravenously.

The febrile responses to daily injections of the same dose of vaccine were recorded on 40 rabbits during periods of from 8 to 45 days. The first injection of vaccine always caused a rise in body temperature of 4-5°F, and some fever persisted throughout the 7-hour period of observation. The 2nd and 3rd injections generally caused almost as much fever as the first, but after that there was a decrease in reaction until the 6th to 10th day. Additional injections caused no further diminution, each one inducing approximately

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[1] Heyman, A., *Ven. Dis. Inform.*, 1945, **26**, 51.

† This vaccine was prepared in the laboratories of the Georgia State Department of Public Health. It is ordinarily used for human immunization and fever therapy.