A STUDY OF ROUS SARCOMA TISSUE GRAFTS IN SUSCEPTIBLE AND RESISTANT CHICKENS

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The object of this investigation was to ascertain the sequelae of grafts of Rous chicken sarcoma placed subcutaneously in normal and resistant pure-bred barred Plymouth Rock chickens.

Rous and Murphy placed small pieces of Rous sarcoma subcutaneously in normal barred Plymouth Rock chickens, and removed them at intervals along with the surrounding tissue. From microscopic study they found that the central cells of the Rous graft underwent necrosis, but the peripheral cells lived, proliferated, and gave rise to neoplasms that eventually killed the bird. In birds having acquired immunity, the findings varied. In some all the cells of the graft became necrotic and no tumor resulted, while in others the peripheral cells gave rise to tumors that eventually regressed and disappeared. In all cases Rous and Murphy were of the opinion that neoplasms resulting from grafts of Rous chicken sarcoma arose from graft cells that lived and proliferated.

A graft of Rous chicken sarcoma removed with the surrounding host tissue presents certain difficulties in histological interpretation. Since the proliferating fibroblasts of the host so closely resemble the tumor cells, there is no definite line of demarcation between graft and host tissue. This junction is also obscured by the presence of great numbers of infiltrating cells.

To obviate this difficulty it was proposed to enclose the graft in some type of membrane, permeable to tissue fluids but impermeable to migrating cells, in order that the origin of proliferating cells could be identified. Various membranes were tried, namely, fresh hen peritoneum, formalin-fixed hen peritoneum, alcohol-fixed hen peritoneum impregnated with metallic silver, egg yolk and egg shell membrane, fixed sheep-cecum sausage membrane, gold beater's skin, and Elford's "gradacol" collodion membranes. Of these membranes, the fixed hen peritoneum impregnated with metallic silver was found to be most satisfactory. This membrane was readily permeable to albumin solutions but impermeable to infiltrating cells. The silver deposit greatly facilitated microscopic identification.

The technic of sac grafting was as follows.

(1) Silvered peritoneum: Sheets of peritoneum from a thin normal hen, freshly killed, were placed in aqueous 2 per cent silver nitrate for

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a few minutes, then spread out and exposed to ultra-violet rays until the peritoneum was uniformly brown. It was then thoroughly washed in several changes of distilled water to remove the excess silver nitrate, placed in saline to precipitate any trace of silver nitrate, and then removed to 70 per cent alcohol for fixation, sterilization, and storage. Prior to use the membrane was thoroughly washed in several changes of sterile saline.

(2) Sac Graft: Small pieces of tumor were punched out by means of a 3 mm. cannula, and cut in 3 mm. lengths. A sheet of silvered peritoneum was placed on an intact sheet of mesentery, left in situ in a freshly killed hen, and the graft placed on the silvered peritoneum. With suction the graft was pulled into a glass tube, shaped to enclose the graft and its surrounding membranes; the neck was tied with white silk and the excess membrane cut away.

(3) Insertion: The graft sacs were placed subcutaneously in the breast region of the hens. A piece of the silk tie of the sac was left protruding through the skin to effect removal. Four sacs in each breast were found to be a convenient number.

Histological Technic: When removed, the sac grafts were immediately placed in 10 per cent formo-saline. They were cut in paraffin, in serial section, and stained with hematoxylin and eosin.

Histological Reaction in Sac Grafts Placed in Susceptible Birds

(1) Fresh Peritoneal Sac Grafts: In all the fresh peritoneal sacs the flattened peritoneal cells took no part in the reaction, as they were not seen even in twenty-four-hour sac grafts. The fibrous stroma of the peritoneum formed the sac membranes in all peritoneal sacs.

After 24 hours, the graft, cut in cross-section, presented a well-staining peritoneal sac fairly well demarcated and containing numerous infiltrating cells. These cells did not resemble polymorphonuclear or lymphocytic leukocytes but possessed a moderately large blue-staining nucleus and a relatively large amount of eosinophilic cytoplasm. The authors believe these cells to be tissue histiocytes.

A dense wave composed of polymorphonuclear and lymphocytic leukocytes was seen to be infiltrating the graft tissue, from its periphery. This wave appeared as a compact circular zone that had advanced equally towards the center of the graft. Between this infiltrating wave and the peritoneal sac was a zone occupied by rapidly dividing fibroblasts, which showed numerous mitotic figures, though some cells were apparently dividing amitotically. The parent cells of the proliferating fibroblasts were not altogether definite, but appeared to be either the reticulum cells of the peritoneal sac or the infiltrating histiocytes or both. The graft tissue central to the infiltrating wave showed well-staining cells, but no evidence of cell division.

At 2 days, the peritoneal sac was intimately fused with the zone of proliferating fibroblasts, which was increased in thickness. Many capillaries were present in this fibroblastic tissue. The infiltrating wave
was well advanced towards the center of the graft, of which the central cells stained well. This advancing wave evidently destroyed all the graft cells it had passed over, as between it and the zone of proliferating fibroblasts there was a zone, formerly occupied by tumor cells, that now was devoid of any tumor or proliferating cells and was occupied solely by leukocytes.

At 3 days, the infiltrating wave had almost reached the center of the graft. The cells of the uninvaded graft were pale-staining. The zone of proliferating fibroblasts was greatly thickened and well vascularized, giving the appearance of rapid growth throughout. The fibroblastic proliferation had also extended peripherally into the adjacent host tissue. This rapidly growing tissue was morphologically identical with Rous sarcoma.

At 4 days, the infiltrating wave had reached the center of the remainder of the graft. Only a few disintegrating tumor cells remained. The zone of fibrous proliferation was now much thickened, and showed increased infiltration of the surrounding host tissue. From the fourth day on, the newly formed tumor tissue increased greatly in amount, invaded the surrounding tissues, and eventually killed the host.

To summarize the findings in the fresh peritoneal sac grafts placed in susceptible birds:

1) There was no evidence of survival of the transplanted tumor cells.

2) There was destruction of the transplanted tumor cells by the leukocytes of the host.

3) The fibroblasts of the host adjacent to the graft became malignant.

There was a possibility that the tumor cells of the graft might migrate through the fresh peritoneal sac into the host tissue, to proliferate, giving rise to tumor. To settle this point, the silvered hen peritoneum was used as sac material and, as it was impermeable to invading leukocytes, it was held that the same impermeability obtained for the tumor cells of the graft.

2 Silvered Peritoneal Sac Grafts: The same technic as used with the fresh peritoneal sac grafts was used for the silvered sac grafts, with the exception that a fresh peritoneal sac was placed external to the silvered sac, for physical support.

The study of the histogenesis of the tumor cells was greatly facilitated by the silvered sac, as it formed a barrier to cell migration and was easily identified microscopically. The cells of the tumor graft stained well up to a period of two days, but from this period on, the staining quality was rapidly lost and the entire graft appeared necrotic. No evidence of cell division or survival was seen in the graft tissue. The infiltrating wave of leukocytes passed through the fresh peritoneal sac but was stopped by the silvered sac in all cases. This wave reached a maximum at two days and then the component cells degenerated. Fibroblastic proliferation was first seen at two days either in the body of the leukocytic wave or in the interstices of the fresh peritoneal sac.
If the fresh peritoneal sac was omitted, early cell proliferation was seen in the body of the leukocytic wave or in the adjacent tissue of the host. This proliferating tissue increased rapidly, became vascularized, and invaded the surrounding host tissue to form typical Rous sarcoma that proved fatal to the host.

To summarize:
(1) All the tumor cells of the graft became necrotic.
(2) All resulting tumor cells arose from cells of the host.

**Histological Reaction in Sac Grafts Placed in Birds Having Acquired Resistance**

In birds having acquired resistance to Rous sarcoma, fresh and silvered peritoneal sac grafts were used in the same manner as in the susceptible birds. The histological reaction presented by the sac grafts placed in such birds was the same as in susceptible birds, with the exception of the zone of fibroblastic proliferation, which morphologically was identical with Rous sarcoma. In one bird this zone was only microscopic in size, and after two days ceased to proliferate, became less cellular, and resembled normal connective tissue. In other birds these proliferating zones increased to macroscopic size, but all such quickly regressed and took on the appearance of normal connective tissue.

**Transplantation of Sac Grafts from Resistant to Susceptible Birds**

Rous sarcoma sac grafts were placed in resistant birds and removed at intervals to normal birds in order to ascertain if such grafts were still capable of giving rise to a malignant tumor. Eight fresh peritoneal sac grafts were placed subcutaneously in the breast of two resistant birds and a sac was removed at daily intervals and transplanted into normal birds. Six silvered hen peritoneum sac grafts were similarly placed in two other resistant birds, and a sac was removed at intervals of two days and placed in normal birds. Of the 20 sac grafts which had remained in resistant birds for more than forty-eight hours, only one produced a tumor when transplanted into normal birds. Of the six sac grafts which were removed from resistant birds in forty-eight hours or less, only two produced tumors in normal birds. The normal chickens which did not develop tumors following sac transplantation from resistant birds were subsequently given Rous extract and all died of tumor.

From these experiments it may be concluded that neither the tumor-producing substance nor the cells in the fresh peritoneal or silvered peritoneal sac grafts survived more than forty-eight hours in resistant birds.
CONCLUSIONS

(1) In both susceptible and resistant birds all the tumor cells of Rous sarcoma sac grafts became necrotic.

(2) In susceptible birds the host cells surrounding the sac grafts gave rise to fatal tumors.

(3) In resistant birds small amounts of tumor-like tissue formed adjacent to the sac graft; this tissue failed to take on malignant characteristics and subsequently regressed.

(4) Cells and tumor-producing substance in the sac grafts did not survive more than forty-eight hours in resistant birds.

REFERENCE