Rous Sarcoma in Folic Acid-deficient Chicks
Morphology and Bioassay*

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INTRODUCTION

Rous sarcoma in chicks which were on a folic acid-free diet, or which received certain of the folic acid antagonists in food or by parenteral injection, was either prevented or markedly inhibited (5). The general pathology of chicks (6) and rats (7) with diet- or antagonist-induced folic acid deficiency was then investigated. The chief changes were in the bone marrow and the gastrointestinal tract. In the marrow there was a transient maturation arrest of all blood cell elements at the level of the stem cells with megaloplasia. This was followed by a total and profound aplasia. In the intestine there was, initially, a marked hydropic swelling of the mucosal epithelium which involved both cytoplasm and nucleus (Figs. 1 and 2). This progressed to severe atrophy with associated ulceration and hemorrhage. The stomach invariably escaped this process. In the chicks the above changes were the same in the dietary or antagonist-produced deficiency, and recovery was seen when the deficient diet was supplemented with the vitamin.

The purpose of this investigation was: (*a*) to compare the microscopic anatomy of the control and the inhibited Rous sarcoma; (*b*) to compare this to the marrow and intestinal changes seen in chicks with folic acid deficiency; and (*c*) to measure, by means of a bioassay, the folic acid content of the tumor and of the host tissue in birds on control, folic acid-free, folic acid antagonist-containing diets and a folic acid-free diet supplemented with folic acid.

MATERIALS AND METHODS

A homogenized sample of Rous sarcoma cells was prepared by means of a Waring Blendor and Ten Brock grinder. A suspension containing approximately 10 mg. of tumor/0.25 ml of saline was then passed through a Berkefeld filter. One-half of 1 cc. of the filtrate was injected into the pectoral muscles of 120 1-day-old New Hampshire Red chicks. The down of the injection site had been removed previously. The birds of this series were divided into groups of 25–30 and were placed on the following regimes.

Group 1.—Control, a commercial baby chick feed.

Group 2.—A synthetic folic acid-free diet (its composition was described in a previous report [5]).

Group 3.—A commercial baby chick feed, as above, plus 80 mg. of 4-aminopteroylaspartic acid mixed into each kilogram of feed.

Group 4.—A synthetic diet, as above, plus a daily intraperitoneal injection of 100 μg. of folic acid in aqueous solution.

A second series of 80 birds, on the same dietary regimes, was divided into 2 sub-groups of 10 each. One sub-group was inoculated with the tumor filtrate as above. The other received an injection of 0.5 cc. of saline only.

Two to four birds from each of the groups in the first series were decapitated on the 2d, 3d, 4th, 7th, 10th, 15th, and 20th days of the experiment, and an autopsy was performed immediately. Multiple blocks of the tumor or of the injection site, as well as of the viscera, were taken for microscopic examination. The tissue was fixed in formaldehyde-Zenker solution, imbedded in paraffin, and stained with hematoxylin and eosin, phosphotungstic acid-hematoxylin, and Schorr's modification of Masson's trichrome stain.

All the animals of the second series were sacrificed on the fifteenth day of the experiment and were examined at autopsy immediately. The tumors were dissected out with care, to exclude nonsarcomatous tissue. The pectoral muscles of

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the saline-injected animals were also excised. The yield from each sub-group was pooled and stored in separate containers in dry ice until used. Weighed equal suspensions of the material were then assayed, with *Lactobacillus casei* as the test organism and folic acid as the standard. The method was that described by Teply and Elvehjem (4), except that Norit-treated peptone and DL-alanine were omitted from the basal media.

RESULTS

Series 1

Group 1 (*commercial feed*).—Every bird showed a rapidly growing tumor with the characteristic infarction, hemorrhage, and myxomatous consistency. Microscopically, the typical cell was small, round, with a delicate, scant, homogeneous cytoplasm, and finely distributed nuclear chromatin (Fig. 3). The cells were arranged in syncytoid masses. Mitoses were infrequent. Little or no reactive change was seen at the margins of the growth. Microscopic pulmonary metastases were found frequently. These showed the same features as the primary tumors.

Group 2 (*folic acid-free diet*).—For the first 7 days, there was no gross evidence of tumor. However, multiple sections of the inoculation site, at many levels, did reveal minute microscopic foci of tumor as early as the 3d day. Later in the experiment (15–20 days), a rare, very small, pale yellow, firm tumor nodule was detected. This is at variance with a previous observation (5), based on gross examination, that no tumor was found in chicks on a folic acid-free diet.

Microscopically, from the earliest evidence of tumor to the end of the experiment, a uniform pattern could be discerned. As compared to the control, the typical cell was much larger; it was round or polygonal. The cytoplasm was more abundant and dense. Centrally, it was coarsely granular, while the margin was vacuolated. The nucleus also was much larger. It was vesicular and contained a single dense nucleolus. Mitotic figures were seen with the same low frequency as in the controls. Phagocytosis by the tumor cells was common (Fig. 4). The older tumor (20 days) showed active proliferation of fibroblasts and capillaries. Collagen deposition was limited to the normal-appearing young fibroblasts. There was also a peripheral accumulation of lymphocytes. Unexpected were two instances of microscopic pulmonary metastasis. The same pattern as in the primary tumor was seen, except for the lack of reactive changes.

Group 3 (*commercial feed with antagonist supplement*).—The outstanding finding was the almost complete absence of tumor, grossly or microscopically. From the fifteenth day on, three instances of a single microscopic focus were found. The pattern was identical in every respect to that seen in the diet-deficient group.

Group 4 (*folic acid-free diet plus folic acid by injection*).—The tumor was recognized on inspection as early as 2 days after inoculation. Growth was distinctly more rapid and voluminous than in the control animals. The microscopic appearances were essentially identical to the controls, except in three animals sacrificed on the tenth, fifteenth, and twentieth days. In these there was little necrosis or hemorrhage in the tumor. It was firm and lobulated. The predominant histologic feature was compact fascicles of well preserved spindle cells with long delicate processes. The small round cells were also present but only in the few soft and partly liquefied areas.

It may be emphasized that the above features were not exclusive in every detail. Individual cells, representative of each of the types described, could be found in any of the groups studied. On the other hand, the predominance of one pattern was strikingly constant for each group.

A comparison of the cytologic alterations in the bone marrow and intestine of folic acid-deficient animals with those of chicks with inhibited Rous sarcoma revealed the following common features: a marked hydropic swelling of the cell (nucleus and cytoplasm), loss of chromatin detail, and arrest of growth. As is well known, this alteration, as judged by conventional stains, is not specific but is rather a common morphologic characteristic of various cellular disturbances. Levine (1), in his thorough investigation of the cytology of Rous sarcoma, described and illustrated the above noted large cells of slowly growing tumors in naturally resistant birds. Similar observations were made by Rous and Murphy (7). We have observed the same phenomenon in a few of a group of older birds, but never in baby chicks.

Series II

Bioassay.—The findings of this procedure are shown in Chart 1. It is to be noted that the tumor contains substantially more folic acid than the pectoral muscle—the tumor site. In the presence of dietary folic acid deficiency, the concentration in the muscle is markedly reduced, while the high level in the tumor is maintained. On a folic acid-supplemented diet, there is increased concentration in both muscle and tumor. However, the amount in the tumor is still relatively higher. In the presence of an antagonist the bioassay fails as a measure of folic acid content.

These findings are in accord with the observations of Pollack (2), who, in a bioassay of a large series of human, rat, and mouse tumors, found the concentration of folic acid to be high in contrast to the other B vitamins.

BIOASSAY OF FOLIC ACID CONTENT		
DIET	FOLIC ACID MICROGRAMS / GM.	
	MUSCLE	TUMOR
COMMERCIAL MASH	0.0123	0.0250
FOLIC ACID-FREE	0.0030	0.0244
FOLIC ACID-FREE PLUS F.A. 10 MGM/KGM./DAY	0.0242	0.0409
COMMERCIAL MASH PLUS 80 MGM/KGM./DAY 4-AMINO-PTEROYL ASPARTIC ACID	INHIBITED	INHIBITED

CHART 1.—Folic acid content of Rous sarcoma tissue and of the pectoral muscles (the tumor site), as determined by bioassay, in chicks maintained on control, folic acid-free, folic acid antagonist-containing and folic acid-free diet supplemented with folic acid.

SUMMARY AND CONCLUSIONS

1. The morphology of Rous sarcoma in chicks made folic acid-deficient by means of diet or antagonist was compared to the alterations in the bone marrow and gastrointestinal tract.

2. Similar comparisons were made with birds on a control diet, as well as on a folic acid-free diet supplemented with folic acid.

3. The folic acid content of the tumor tissue and of the pectoral muscle (the tumor site) was determined by means of a bioassay in animals on the above regimes.

4. Folic acid deficiency is associated with marked decrease in the growth of Rous sarcoma.

5. This is characterized by a marked increase in the size of the tumor cell (nucleus and cytoplasm), hydropic swelling of the nucleus, and apparent concentration of the chromatin.

6. These features are also seen in the immature cells of the bone marrow and the gland cells of the intestine of the deficient animals.

7. Rous sarcoma contains considerably more folic acid than the tissues of the tumor site. This relationship is maintained even in the presence of folic acid deficiency or folic acid excess in the diet.

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FIG. 1.—Normal chick duodenum. Hematoxylin and eosin. ×450.

FIG. 2.—Chick duodenum after 7 days on a folic acid-free diet. Note the swollen cells, loss of chromatin detail, and disrupted configuration. Hematoxylin and eosin. ×450.

FIG. 3.—Rous sarcoma in a control bird (15 days). Hematoxylin and eosin. ×450.

FIG. 4.—Rous sarcoma in a bird after 15 days on a folic acid-free diet. Note the marked swelling of the tumor cells, loss of chromatin detail, and hydropic swelling of the nucleus. Hematoxylin and eosin. ×450.