Neurofibromas of Rat Ears Produced by Prolonged Feeding of Crude Ergot

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As a result of an experiment to determine the chronic toxicity of crude ergot when fed to our inbred strain of Osborne-Mendel albino rats for 2 years, 23 of 38 rats on a 5 per cent dosage developed multiple tumors up to 8 mm. in diameter on their ears. Tumors appeared also on the ears of 9 of 38 rats on a 2 per cent dosage, but not on any of 38 on a 1 per cent dosage.1 Histologically these tumors are neurofibromas, some showing distinct palisading of nuclei. They have appeared only on the ears. Male and female rats are affected alike. Among several hundred of our rats allowed to live normally or subjected to other experimental procedures, and whose ages equal those of the rats fed ergot, only one fairly similar tumor has appeared; this tumor was also on an ear. The tumors induced by the feeding of crude ergot are not truly malignant, inasmuch as they will regress markedly within a few weeks if feeding of the drug is stopped. Should the feeding be resumed they will then regain their former size and histologic appearance in 2 or 3 months. If the feeding is not resumed, some of the tumors will begin a spontaneous new growth after about 6 months.

MATERIALS, METHODS, AND RESULTS

Source of ergot.—The ergot used by us was a mixture of a dozen or more lots of imported ergot pooled to give a total of about 40 pounds of crude ergot, which has lasted throughout the experimental work reported here. Crude ergot loses its activity very slowly, as measured either by the U. S. P. cock's comb method or by colorimetric assay for total alkaloids, if it is kept dry in a tightly covered container. Ground ergot deteriorates more rapidly (1), and therefore we ground small lots when needed.

Experimental procedure.—Groups of 20 rats each were started at 3 weeks of age on dosages of 5, 2, and 1 per cent of ground crude ergot in their diet; the rest of the diet consisted of ground dog-food pellets plus 1 per cent cod liver oil. Probably because it was not learned until later that rats would tolerate 5 per cent of ergot better if the dosage were increased gradually, 11 of this group of rats died within 1 year. Three other rats in this group died at 14, 18, and 22 months of age; then, in a rat dying at 23 months of age (in May, 1940; Pathology No. 308) it was noted that several soft nodules up to 4 mm. in diameter were present on the ears. On microscopic examination they were diagnosed as fibromas, most likely of perineural origin. The remaining 5 animals in the 5 per cent group, dying at greater ages or surviving the 2-year experimental period, all had similar tumors on the ears, with a similar microscopic appearance. It is quite possible that the 2 rats dying at 18 and 22 months of age had small car tumors which had not been noticed. Of the groups of rats fed 2 per cent and 1 per cent of ergot, 16 of each 20 lived from 18 to 25 months; 3 rats on the 2 per cent dosage and none on 1 per cent had car tumors.

While these experiments were in progress and before the ear tumors had appeared, a second series of experiments was begun, with the same levels of ergot in a low protein diet; there were 18 rats in each of these 3 groups. When the rats of the second series were 6 months of age, ear tumors were seen in the animals of the first series. When rats of the second series were 11 months of age, ear tumors began to appear in the group fed 5 per cent ergot; they continued to appear until at 17 months of age every one of the 17 living rats had ear tumors. Microscopic sections of these tumors showed the same neurofibromatous structure as in the first series. At the time of writing this report (October, 1941) tumors have also appeared on the ears of 6 rats in the 2 per cent group and on none in the group receiving 1 per cent ergot. These rats are now 23 months of age. The low protein diet of the second series of rats seems to have increased somewhat the size and number of the tumors and to have caused them to appear earlier than those in the first series.
Gross appearance of tumors.—Grossly the tumors are rounded, circumscribed but not encapsulated, moderately firm subcutaneous masses on the inner, outer, or both surfaces of the ears. They are greatest in number at the periphery and least frequent toward the external meatus. The number on each ear varies from 1 to 8 (Fig. 1). On section the tumors are pinkish-white and rather pearly. The smaller tumors are uniform throughout; the larger ones often show ulceration and crusting of the surface (Fig. 2), and occasionally show internal areas of loose texture and slightly darker color than the rest of the tumor. The maximum size reached, after several months of growth, is 8 mm. or slightly greater in diameter. When an ear has several tumors of this size the tumors almost coalesce, and the aggregate size tends to remain fairly constant through ulceration and drying of portions of the tumors.

Routine microscopic sections of the major viscera of these rats, those without ear tumors as well as those with them, have shown that the tumors occur only on the ears, and that they do not metastasize.

Microscopic appearance of tumors.—The microscopic appearance of the tumors is quite uniform, and is characteristic of neurofibroma. The tumors are composed of oval to spindle-shaped cells of medium size, with leptochromatic nuclei and little neutrophilic cytoplasm, often arranged in interlacing whorls (Fig. 5) and fairly often in palisade formation (Fig. 3). Mitoses are few in number, varying from about 1 to 10 per square millimeter of tumor section. Small nerves containing both myelinated and unmyelinated fibers are frequent among masses of tumor cells (Fig. 4). The frequent presence of nerves within tumor tissue and of nuclear palisading leaves little doubt that these tumors are neurofibromas.

Further support of the microscopic diagnosis is given by various selective stains. The Masson and Van Gieson types of fiber stains show few collagenous fibers. With silver impregnation a dense reticulum of argentophilic fibers is seen. Frozen sections stained with Sudan IV show no fat in the tumor cells; a small amount is present in the macrophages in ulcerated areas. Sections stained with osmic acid show a similar picture. These macrophages also contain a small amount of brown pigment, some of which reacts for ferric iron (hemosiderin) with acid ferrocyanide. There is no pigment in the tumor cells.

The tumors will grow through the naturally occurring holes in the cartilage plate of the ear, and will also cause more or less pressure atrophy of the cartilage and of the adjacent striated muscle.

Regression and reappearance of tumors.—It occurred to us to try the effect of stopping the feeding of ergot to rats with well established tumors. Truly malignant induced internal tumors, such as the liver carcinomas resulting from the feeding of ortho-aminoazotoluene to rats, will keep on growing after the feeding of the carcinogen is stopped (5, 7). As no peripheral tumors other than the neurofibromas described in this report have as yet been produced by feeding a substance, we have no basis of comparison. The feeding of ergot was therefore stopped for 9 rats on the 5 per cent level, all of which had ear tumors. Three weeks later most of the tumors showed a marked reduction in size. After 3 more weeks there was a further slight reduction, so that the former typical tumors of about 8 mm. diameter were now about 4 mm. across and 1 mm. in height, while some of the smaller tumors had practically disappeared.

Six weeks after it had been discontinued, the feeding of 5 per cent ergot was resumed for 4 of the 9 rats. The tumors on the ears of these 4 rats remained about as described above for 2 months, but at the next examination a month later there had been marked growth, so that 3 months after the resumption of ergot feeding the tumors on 3 of the 4 were almost the size previously attained, while the fourth, which had small tumors to begin with, showed slight growth. Further growth took place later. Eventually some of the tumors were larger than before the animals had been taken off the 5 per cent ergot diet.

Meanwhile, the tumors on the 5 rats for which ergot feeding was not resumed showed a slow, progressive decline in size. At 5 months, partly because the larger remaining tumors had been removed for microscopic study, there remained only flat brown spots up to 3 mm. in diameter. These were best seen by transmitted light. However, at 6 months a new development was noted. Four of the 5 rats died about a week apart, and on an ear of one of these was a new 4 mm. tumor.

DESCRIPTION OF FIGURES 1 TO 5

Fig. 1.—Gross appearance of multiple and bilateral tumors on the ears of rats Nos. 829, 853, and 863.

Fig. 2.—Photomicrograph of a longitudinal section through one of the larger tumors, from rat No. 1661, showing ulceration of the overlying epidermis, and a small extension of the tumor beneath the cartilage plate of the ear. Hematoxylin and eosin stain. Mag. X 18.

Fig. 3.—Photomicrograph of tumor in rat No. 830, showing whorl formation and cellular details. Hematoxylin and eosin stain. Mag. X 225.

Fig. 4.—Photomicrograph of a tumor in rat No. 864, showing palisade arrangement of nuclei. Hematoxylin and eosin stain. Mag. X 575.

Fig. 5.—Photomicrograph of area in a neurofibroma in rat No. 864, showing palisade arrangement of nuclei. Hematoxylin and eosin stain. Mag. X 720.
with the typical histologic appearance of these tumors. Also, the one rat still alive, after having had no ergot for 7 months, showed a new 2 or 3 mm. tumor. It would appear, then, that the residues of the tumors left after ergot feeding is stopped can grow spontaneously after a latent period of several months. The difficulty here is that the regrowth begins to take place near the end of the life span of the rat.

At intervals during the course of study of regression and reappearance of these tumors, several of them were removed for microscopic study. Three weeks after discontinuing ergot one tumor showed, in addition to the decrease in size, less active tumor tissue, in which were a moderate number of macrophages containing fat as shown with Sudan IV, and fewer containing brown pigment, chiefly hemosiderin as shown with acid ferrocyanide. At 4 months, in 5 tumors removed from 2 rats, various stages of regression were present. Two tumors were essentially as just described; another showed marked regression, appearing as a mass of loose, faintly mucoid fibrous tissue, with very little fat or pigment. The remaining two tumors, although small, had practically the same histological appearance as in the animals still on ergot. Collagen and reticulum stains in the regressing tumors showed these components to be present in about the same relative degree as before; the total amount was, of course, decreased. At 6 months, in the tumors showing no regrowth, there was only a little collagenous thickening, a little grayish-brown pigment in macrophages, and few or no tumor cells.

Rous and Kidd (8) have recently discussed the regression of the tumors on the ears of rabbits when application of the tar is stopped.

**Neurofibromas and Other Spontaneous Tumors in Rats Not Fed Ergot**

In about 1,000 rats of all ages, not fed ergot, and studied histologically concurrently with those fed ergot, only one neurofibroma has occurred. This was on the ear of a rat 20 months of age and measured 7 x 7 x 3 mm. Histologically it was slightly malignant and was classed as a grade 1 neurofibrosarcoma; it still retained the whorl formations of a neurofibroma. In the literature on rat tumors, both spontaneous and induced, we have been unable to find any diagnosed as neurofibroma or neurosarcoma. It is possible, however, that some of those diagnosed as fibroma or fibrosarcoma are in this class.

The feeding of ergot did not increase the incidence of the usual assortment of spontaneous tumors and leukemias to which the rat is subject, and which have been carefully studied for our colony. The rats fed ergot have had the usual number of these conditions, and no more, even among the group of animals bearing highly malignant fibrosarcomas which many believe have a neurogenic origin (2).

**Pathological Changes Other than Tumor in Rats Fed Ergot**

A report on the visceral lesions in these rats will be included later in a paper dealing with the pharmacological aspects of this study. To summarize briefly, however, it may be stated here that two other lesions specifically caused by the ergot were frequently observed. The first was necrosis and calcification of the lower end of the renal medulla, more marked in the groups on the low protein diet, although control animals on a low protein diet never had this lesion. We have never seen or read of this lesion in rats; it is found in some mice (3). The ovaries were frequently enlarged and mulberry-like, and composed chiefly of corpora lutea, similar to the appearance produced by chorionic gonadotropin; none of our other rats have shown this. Another, but nonspecific, lesion was a stunting of growth proportional to the dosage of ergot.

No vascular or cardiac lesions attributable to the ergot were found, and no gangrene occurred. Such experimental lesions as have been produced along these lines have usually been obtained by injection of ergotamine tartrate (6) in doses far greater than present in our crude ergot.

We have seen no report of the feeding of crude ergot for more than 3 months to animals other than roosters (4). A case of human cutaneous carcinoma in which the prolonged application of an ointment containing ergot played an uncertain part (9) has been described.

**Further Investigation**

Our findings raise a number of questions which our laboratory, not being primarily equipped for cancer research, cannot investigate in detail. The chief question concerns the exact fraction of ergot responsible for tumor production. Ergot is a highly complex substance. As space does not permit even a listing of its constituents, the reader is referred to Barger's (1) monograph. Tissue culture and transplantation have not been attempted, nor has ergot been fed to animals other than rats. We invite attempts by others to investigate these points. A rough fractionation study, with the feeding of defatted ergot, fatty residue, and ergotoxine to different groups of rats has been started by us.

**Summary and Conclusions**

1. Histologically typical neurofibromas have been produced on the ears, and on the ears only, of a high percentage of rats by prolonged feeding of 5 per cent of crude ergot in the diet.
2. The tumors have occurred less frequently on a level of 2 per cent of crude ergot and rarely on a level of 1 per cent. A low protein diet somewhat favors the production of tumors.

3. The neurofibromas have been made to regress markedly by withholding ergot, and then to reappear by refeeding.

4. After about 6 months without feeding of ergot, some tumors which have markedly regressed will spontaneously grow again; however, this is practically at the end of the life span of the animals.

5. Two other lesions, a renal medullary necrosis and calcification, and enlargement of the ovaries, are frequently caused by feeding of ergot. No cutaneous gangrene and no vascular lesions attributable to ergot have been observed, probably because the dosage in terms of alkaloids has been much too low.

6. The exact constituent of the crude ergot responsible for the tumor production is not known.

REFERENCES

7. Personal observations in this laboratory.
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