

# STUDIES ON TUMOUR INHIBITORY ACTIVITY OF INDIGENOUS DRUGS

## Part I. Tumour Inhibitory Activity of *Hippophæ salicifolia*, D.DON

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SURGERY and radiations are the two, present-day, methods for the treatment of human cancers, but certain types of cancer are not amenable to these treatments. Hence, a study of the treatment of cancer with chemical agents, alone or in combination with the above methods, has been engaging the attention of scientists during the last fifty years. As a result of their efforts, it is found<sup>1</sup> that some cytotoxic poisons, alkylating agents, antimetabolites, vitamin antagonists, hormones, antibiotics, etc., produce temporary beneficial effects in the treatment of certain types of human cancer.

An important source of drugs which has also been attracting the attention of cancer workers is that derived from plant products. The study of plant products is of special interest to workers in India because of the easy availability of a large quantity of plant materials and a large variety of plants which exist owing to varied climatic and other environmental conditions. Research workers are naturally attracted towards a study of plant products because of the possibility of encountering newer compounds which are difficult to synthesize in the laboratory. Guided by such considerations, three groups of workers from U.S.A.<sup>2-4</sup> have been screening plants against transplantable tumours.

As a result of their aggregate work, about 1,800 plants have been screened so far. It has been found that, colchicine<sup>5-7</sup> and podophyllin<sup>8</sup> cause damage to experimental tumours; while Sanguinarine,<sup>9</sup> Chelarythrine<sup>9</sup> and Vincalokoblastine<sup>10</sup> possess some activity against Ehrlich carcinoma in mice. Thus, studies on plants continue to yield new information which may later be useful for possible trial against cancer in human beings.

A careful study of the plant remedies given in *Sashruta-Samhita* and the current medical literature was made by us and some plants were selected for

evaluating their chemotherapeutic value in cancer. One such plant studied by us in some detail is *Hippophæ salicifolia*, D.DON. The results of the work on the bark of this plant are presented in this paper.

The plant *H. salicifolia*, D.DON is called 'Chuma' or 'Kalabis' in Punjab.<sup>11</sup> It grows in temperate regions of the Himalayas from Kunawar to Kumaon at a height of 7,000 to 11,000 feet.<sup>11</sup> It is a willow-like shrub, 10–20 feet in height. Its leaves<sup>11</sup> are membranous and glabrous. They are 2–4 inches long, dull-green, linear-lanceolate and densely clothed on the under surface with white or rusty stellate hair and some circular scales (Fig. 1). The fruit of the plant has been stated to be of value in lung complaints.<sup>11</sup> Its bark is dark-grey, brown, soft and fairly thick (Fig. 2). Three extracts of the bark (i) Aqueous extract, (ii) Alcoholic extract (A) and (iii) Alcoholic extract (B) were prepared for studying their antitumour activity.

The toxicity of the alcoholic extract (B) was first studied and the findings as regards the dosages were applied in the case of other two extracts for all the extracts ultimately consisted of the water-soluble portion of the powdered bark.

The above extracts were tested on two types of transplantable tumours (1) a slow-growing fibrosarcoma<sup>12</sup> (solid) in Swiss mice, and (2) a fast-growing Yoshida sarcoma (ascites) in Wistar rats, procured through the kind courtesy of Prof. Drukrey from Freiburg. The extracts were administered to mice subcutaneously (i) on alternate days from the 2nd day of tumour transplantation, (ii) on alternate days from the 10th day of tumour transplantation, up to 21 days and (iii) everyday from the 8th day of tumour transplantation until they were weak and about to die. Adequate controls were kept and they were given equal amount of water by the same route.

The results of the screening work on *H. salicifolia* indicate that the extracts of the bark of the plant possess significant inhibitory action on mouse fibrosarcoma (Table I). There is a marked difference in the size of tumours which were observed in the control and experimental animals (Fig. 3).

Table II shows that the percentage inhibition is of a fairly high order. Both the aqueous and alcoholic extracts of the bark exhibit this activity. There is an appreciable reduction in the growth even in the case of an established tumour (Fig. 4).

Prolonged treatment of the alcoholic extract (B), started after 8 days of tumour transplantation inhibited the growth of tumour cells of mouse fibrosarcoma, but complete regression of tumour did not take place.

TABLE I  
*Tumour weights (gm.) from representative batches  
(Mouse fibrosarcoma)*

| No. | Aq. Ext. |      | Alcohol Ext. (A) |     | Alcohol Ext. (B) |      |
|-----|----------|------|------------------|-----|------------------|------|
|     | C        | E    | C                | E   | C                | E    |
| 1   | 1.80     | 0.48 | 5.5              | 2.0 | 1.44             | 0.24 |
| 2   | 2.63     | 0.26 | 6.5              | 3.0 | 1.54             | 0.20 |
| 3   | 2.25     | 1.31 | 6.5              | 2.0 | 2.14             | 0.81 |
| 4   | 3.90     | 0.90 | 4.0              | 2.5 | 1.60             | 0.79 |
| 5   | 3.60     | 1.38 | 4.5              | 2.0 | 2.59             | 0.32 |
| Av. | 2.83     | 0.86 | 5.40             | 2.3 | 1.86             | 0.47 |

C—Control treated with water.

E—Experimental treated with *H. salicifolia* extracts.

TABLE II  
*Inhibition of mouse fibrosarcoma with H. salicifolia*

| Group | Preparation      | Dose c.c. | No. of Injections | Av. Tumour Control | Wt. (gm.) Exptl. | % Inhibition |
|-------|------------------|-----------|-------------------|--------------------|------------------|--------------|
| 1     | Aq. Extract      | 0.2       | 10                | 5.81               | 2.26             | 61           |
|       | Aq. Extract      | 0.2       | 10                | 2.83               | 0.86             | 69           |
|       | Aq. Extract      | 0.4       | 10                | 4.04               | 1.77             | 56           |
|       | Alcohol Ext. (A) | 0.2       | 11                | 5.40               | 2.30             | 57           |
|       | Alcohol Ext. (B) | 0.2       | 9                 | 1.86               | 0.47             | 74           |
|       | Alcohol Ext. (B) | 0.2       | 10                | 3.48               | 1.25             | 65           |
| 2     | Alcohol Ext. (B) | 0.2       | 8                 | 6.73               | 2.29             | 66           |
|       | Alcohol Ext. (B) | 0.5       | 2                 | 6.49               | 2.89             | 56           |

1—Treatment from 2nd day of transplantation.

2—Treatment from 10th day of transplantation.

Histological study of the tumour tissues (Figs. 5 A, 5 B) showed that (i) there was a degeneration and even necrosis of tumour cells in large portions of the new growth while (ii) in the case of well-established tumours, the degeneration and necrosis of cells was less extensive. Several groups of cells appeared to be unaffected and viable.

The alcoholic extract (B) of the bark also showed an inhibitory activity against Yoshida sarcoma (ascites), as evidenced by the increase in survival period of the experimental animals (Table III). The aqueous extract of the bark, however, did not exert this influence to any noticeable extent on Yoshida sarcoma (ascites).

TABLE III  
*Effect of H. salicifolia extracts on Yoshida sarcoma bearing rats*

| Group | Preparation      | Daily Dose I.P. c.c. | Average Survival in days |
|-------|------------------|----------------------|--------------------------|
| E     | Alcohol Ext. (B) | 0.25                 | 8                        |
| C     | Water            | 0.25                 | 4                        |
| E     | Aq. Ext.         | 0.50                 | 3                        |
| C     | Water            | 0.50                 | 3                        |

E—Experimental.

C—Control.

In view of the above results, chemistry of the bark of *H. salicifolia* has been under investigation. From the ether extract of the bark a compound, m.p. 285–86°, has been isolated. Chloroform extract has also yielded a crystalline compound, m.p. 135°. Both these compounds are being accumulated for further studies.

It may be of interest to know that a variety of this plant, *Hippophae rhamnoides* Linn. has been reported to possess antitumour property.<sup>13</sup>

#### EXPERIMENTAL

Authentic samples of the bark of *H. salicifolia* D.DON were procured through the Forest Utilisation Officer (U.P.). The bark was dried in an air-oven at 60° and powdered in a laboratory grinding mill (80 mesh). The procedure employed for the preparation of the three extracts was as follows:

(i) *Aqueous extract* was obtained by refluxing the bark powder (1.0 g.) with distilled water (10 c.c.) for about 15 minutes. It was then cooled, filtered. This extract was prepared fresh every time.

(ii) *Alcoholic extract (A)*.—The powder of the bark (100 g.) was extracted in a soxhlet apparatus with ethyl alcohol (500 c.c.). The extract on concentration gave a residue which was dissolved in distilled water to yield a 10% suspension.

(iii) *Alcohol extract (B)* was obtained by shaking the bark powder (50 g.) with ethyl alcohol (4×250 c.c.) The resulting extract was filtered, concentrated under vacuum in an atmosphere of nitrogen at 60–65°. The residue obtained was treated with distilled water to obtain a 2.5% water-soluble portion of the extract.

*Toxicity Studies*.—Batches of six mice (weighing 18–20 g. each) were injected subcutaneously with doses of 0.2, 0.4, 0.5, 1.0 and 1.2 c.c. of the alcoholic extract (B). The last two doses were found to be toxic as the animals lost weight rapidly and died on the third day of the injection. On autopsy, the lungs and perivascular tissue were found to be hæmorrhagic.

Batches of six rats (weighing 129–149 g. each) were injected intraperitoneally with doses of 0.25, 0.5 and 1.0 c.c. of the alcoholic extract (B). The last dose was found to be toxic as the animals died on the same day of the injections.

*Screening procedure*.—Mice: A group of about 12–16 mice (Swiss inbred strain, 6–8 weeks old) was used for testing the above extracts. A tumour mince (0.2 c.c. of 50% in saline) prepared from a 21-day old mouse fibrosarcoma was injected subcutaneously in animals by means of 18-number needle. They were then divided into two equal batches, one for control and another for treatment with the extract.

*Experiments on mice*.—I. Subcutaneous injections with 0.2 c.c. of aqueous extract were administered to two batches of mice (10 each) on alternative days, while the effect of dose of 0.4 c.c. was studied on a batch of 10 mice. Alcoholic extract (A) was tested on a single batch of mice in the same way. Alcoholic extract (B), however, was tested on two batches of mice. The controls were given an equal amount of water by the same route. Injections were started from the second day of tumour transplantation and on an average ten injections were given.

The animals were sacrificed 21 days after the tumour transplantation, and their tumours were dissected out, weighed and measured (Table I). Histopathological study of the tumour tissues from a batch was done.

II. In another set of experiments, the injections were started ten days after the transplantation of the tumour. Animals having tumours of about the same size were selected for study. The effect of varying doses (0.2 c.c. and 0.5 c.c.) of the alcoholic extract (B) was studied in two batches. The control and the experimental animals were sacrificed in these two batches 21 days after tumour transplantation and their tumours were dissected out, weighed and measured. In one batch, histopathological study of tumour tissues was done.

III. A batch of 18 Swiss mice (6-8 weeks old) was transplanted with tumour mince as usual. The tumour was allowed to grow for 8 days and after its establishment, the animals were divided into 3 equal groups I, II and III. The groups I, II and III were daily given subcutaneous injections of 0.2 c.c. alcoholic extract (B) for 7, 14 and 21 days respectively.

All the animals were carefully observed during the experimental period. Weak animals were sacrificed immediately. Animals from these three groups (I, II and III) were allowed to survive for the maximum number of days and then sacrificed one after another when they looked weak. Their tumours were carefully dissected out, weighed, measured and used for the histopathological study.

IV. *Experiments on Rats.*—A batch of 10-12 rats (Wistar strain, 3-4 months old) was used for testing the aqueous and alcoholic extract (B). The ascitic fluid (0.5 c.c.) from a Yoshida sarcoma bearing rat was injected intraperitoneally into the rats. The animals were divided into two equal groups. The experimental group was treated with extracts and the control group was given an equal quantity of water intraperitoneally on the second day after the transplantation of ascitic fluid. Different doses of the alcoholic (B) and aqueous extracts (0.25, 0.5 c.c./rat/day) were given to rats and both the control and experimental animals were observed till their deaths (Table III).

#### SUMMARY

The extracts of the bark of *Hippophæ salicifolia* D.DON have been found to possess significant inhibitory activity on mouse fibrosarcoma. The tumour tissues showed that there was a degeneration and even necrosis of tumour cells. The alcoholic extract (B) of the bark also possesses an inhibitory activity against Yoshida sarcoma (ascites), as evidenced by the increase in survival period of the experimental animals. The chemistry of the bark is under investigation.

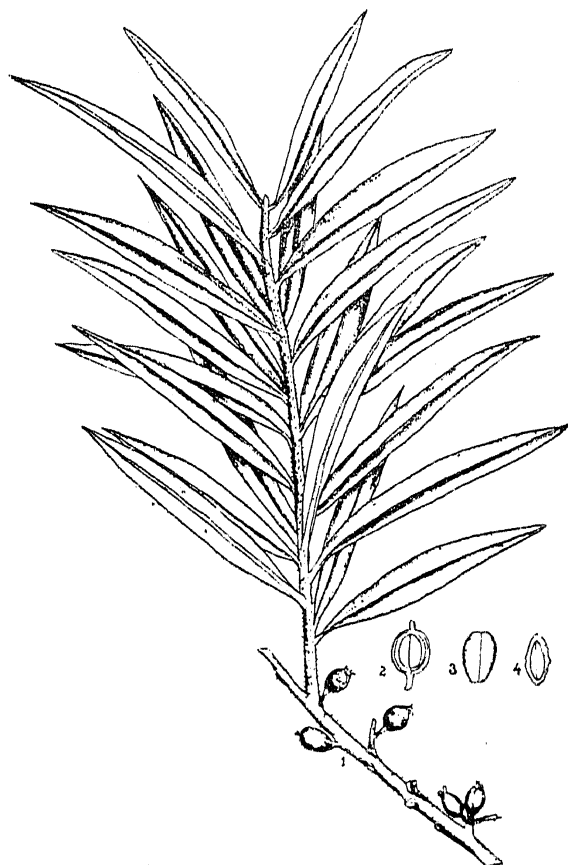


FIG. 1. Plant of *Hippophae salicifolia*.

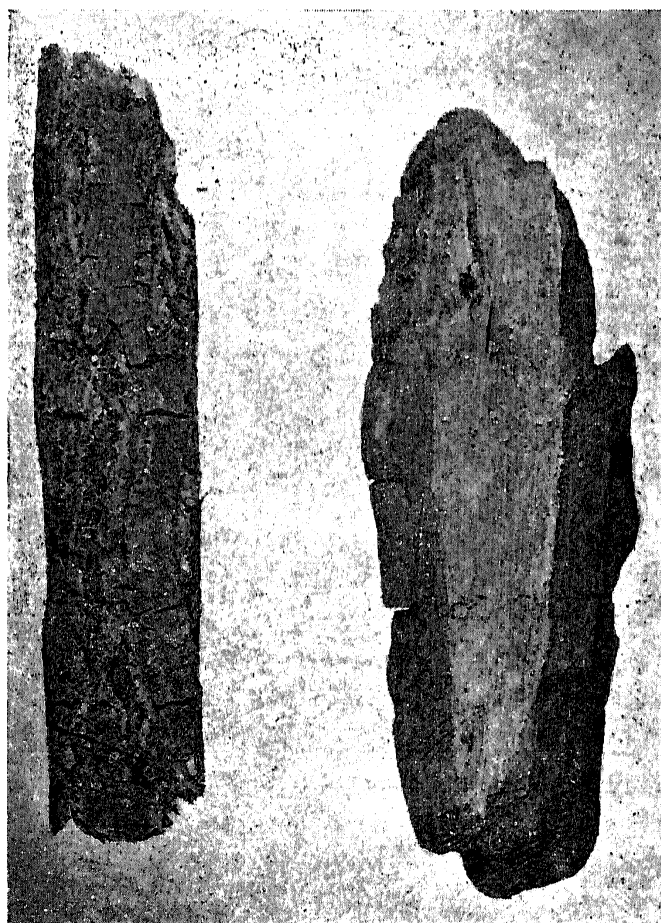


FIG. 2. Bark of *H. salicifolia*.

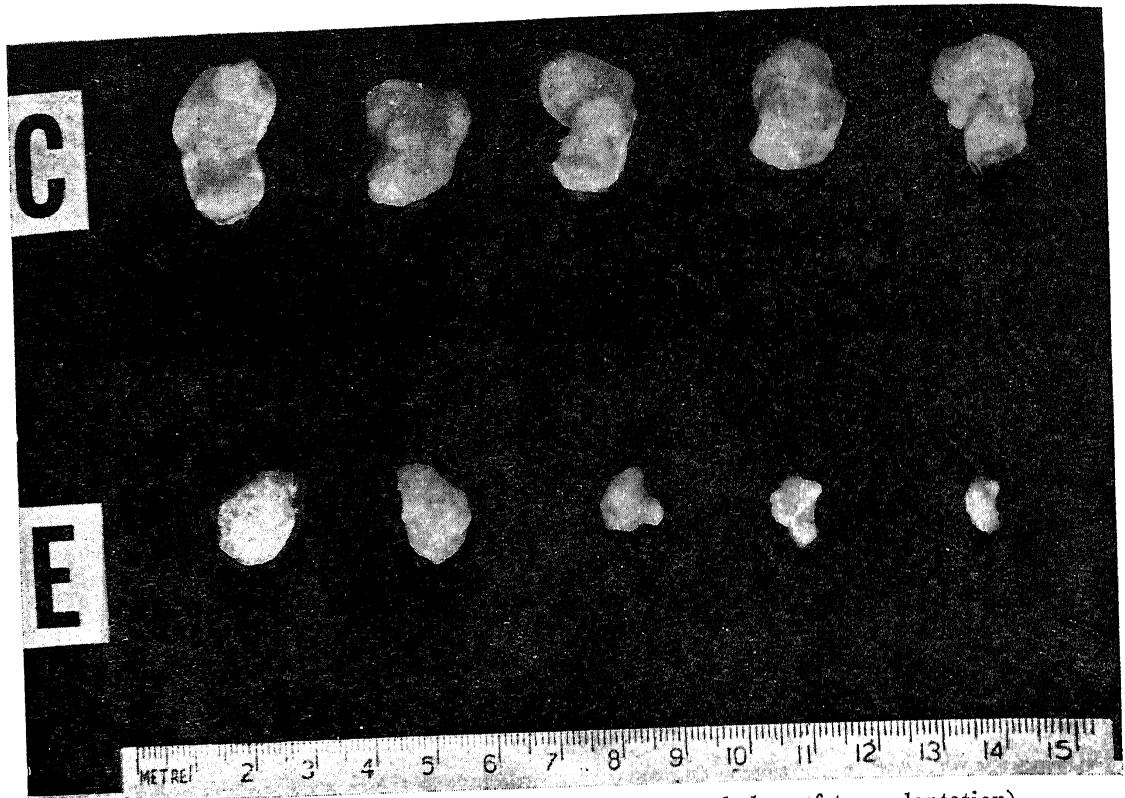


FIG. 3. Inhibition of tumour (Treatment from 2nd day of transplantation).



FIG. 4. Inhibition of tumour (Treatment from 10th day of transplantation).



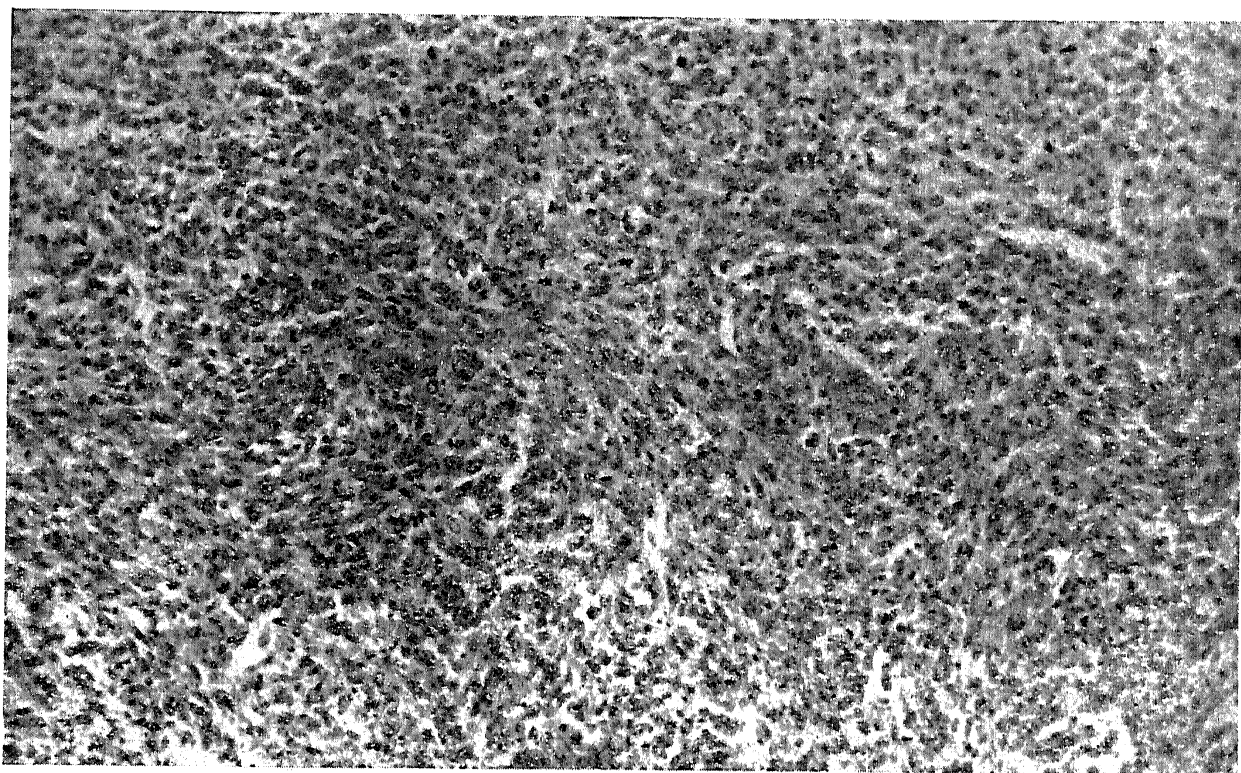


FIG. 5 A. Mouse fibrosarcoma,  $\times 175$ .

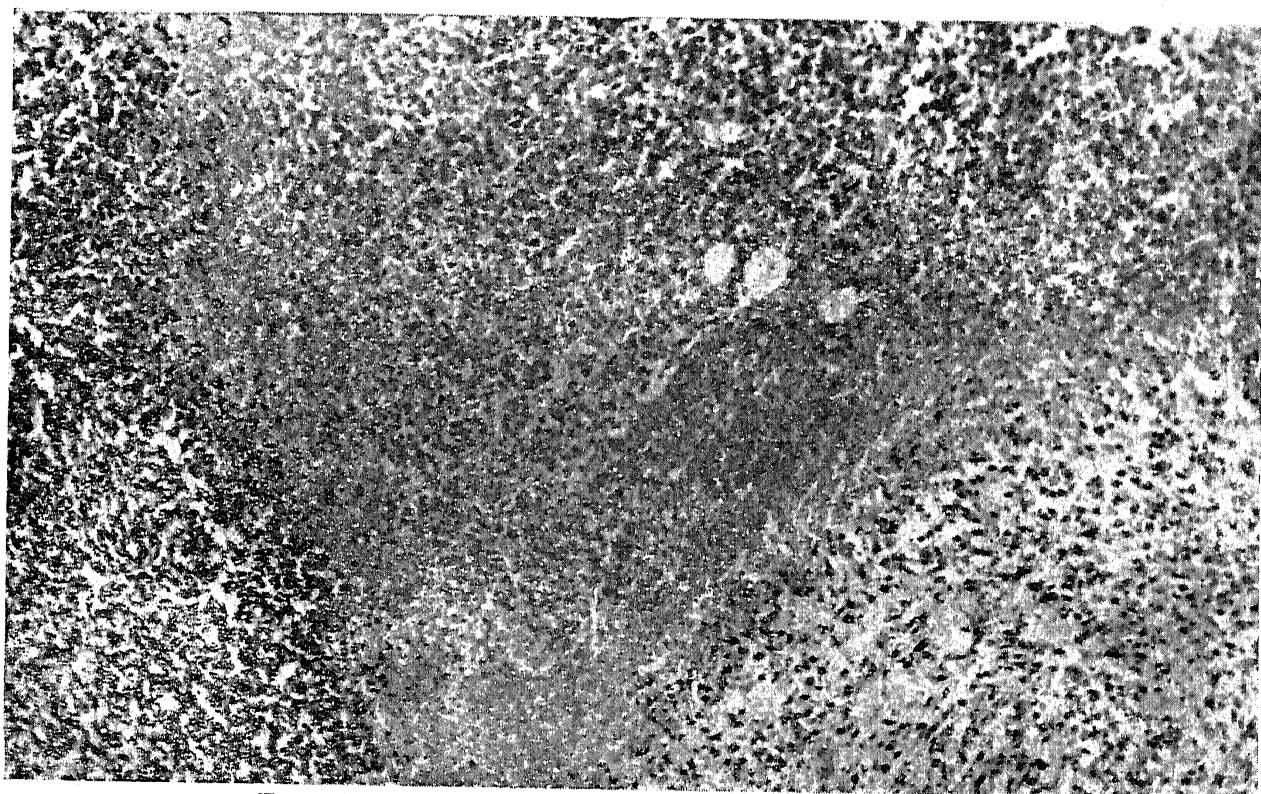


FIG. 5 B. Mouse fibrosarcoma (after treatment),  $\times 175$ .

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