In all cultures, after 48 h, the monolayers were washed and stained and the plaque inhibition titer recorded. The PMN supernatants were also examined for virus neutralizing activity against VSV and IBR by methods described before[5]; no neutralizing activity was found.

The details of the methodology used to produce bovine types I and II interferons were described previously[18]. Also described previously were the methods of measuring acid sensitivity and the antiviral activity of the compounds in different cell lines[18].

Results and discussion. As is shown in figure 1, IBR-virus-infected GBK cells (IBR-GBK) was a potent stimulator for the release of an antiviral inhibitior from the bovine PMN cultures. Thus the cell free supernatant fluid, from such cultures had antiviral activity against both VSV and IBR viruses. The stimulatory effect of the IBR-GBK antigen was dose dependent and was apparently the result of stimulation of PMN by IBR viral induced antigen since uninfected GBK cells failed to cause the release of any inhibitor. As few as approximately 1000 virus-infected cells were needed to cause the PMN cultures to release detectable inhibitor.

The inhibitor was first detectable 6–9 h after stimulation, reached peak levels at 18–21 h and persisted for at least 48 h (figure 2). The activity was expressed against both VSV and IBR viruses. Although virus-infected cells caused the release of large amounts of inhibitor, the levels of free virus released from such cells were insufficient to stimulate release. In fact it was necessary to concentrate virus 10fold or more for it to be stimulatory (equivalent to virus released from approximately 4×10^5 infected GBK cells). Since such cell-free virus could additionally contain cell membrane fragments, it is possible that such fragments, with incorporated virus-coded antigen, were responsible for the stimulation.

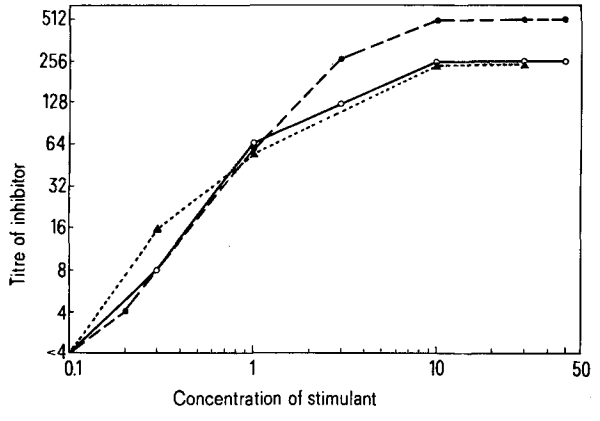

Fig. 2. Kinetics of production of inhibitor of VSV virus replication after stimulation of PMN with UV-irradiated IBR-virus-infected GBK cells. The results of 3 separate experiments are shown.

Whereas GBK cells infected with IBR virus regularly triggered PMN to release the inhibitor, other virus-infected cells tested were unable to do so (table 1). These observations may mean that the PMN bear a receptor that specifically recognizes a herpesvirus. However, many more viruses must be investigated and direct binding studies must be done, before such a claim can be substantiated.

It is of interest that our observation may not merely be restricted to the bovine system, since we have observed, in preliminary experiments, that human PMN can be triggered by Herpes simplex virus infected VeRo cells to release a similar inhibitor (unpublished data). Although the antigen recognition event required for the induction of the inhibitor may prove to be virus-specific, the material itself is, like type I interferon, nonspecific in that the activity was expressed against both DNA and RNA viruses. Also in resemblance ot interferon, the material exhibited no direct antiviral effects as could be determined by virus neutralization assays. However, preliminary characterization studies have revealed differences from bovine type I and type II interferons, in that the material shows an intermediate level of acid sensitivity and antiviral activity in some heterologous cells (table 2).

Studies are in progress not only to characterize the inhibitory material, but also to describe the optimal conditions needed for its production. It seems not to be performed, since sonicates of PMN were not inhibitory (table 2). The contents of PMN granules did not appear to be the inhibitor since treatment of PMN with antibody-complement opsonized zymosan particles, a procedure that causes the release of granule contents[17], failed to trigger the release of the inhibitor (table 1).

Whatever the nature of the inhibitor or the exact conditions needed for its production, our studies do serve to strengthen the hypothesis that PMN may be extremely important effector cells in antiviral immunity. It might also be that we have identified a 3rd, type of interferon – if so we could provisionally name the material interferon 3.

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Foliar spirality and aestivation of flowers in *Hibiscus cannabinus* Linn.

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Summary. In *Hibiscus cannabinus* a negative association exists between the foliar spirality and the aestivation of corolla. Moreover, it is seen that the fruits developed from left-twisting flowers of left-spiralled plants and those of the right-twisting flowers of right-spiralled plants are better in quality.

The aestivation of corolla in *Hibiscus cannabinus* is contorted (the 5 petals twist regularly) as in other species of Malvaceae[2–4]. In about 50% of the flowers on any of these annual plants, the contortion of petals is clockwise (left-handed) and in the rest, counter-clockwise (right-handed). Since the phyllotaxy in this species is alternate (cyclic), the plant/shoot can be grouped into left-handed or right-handed[5]. The foliar asymmetry is not genetically determined. However, there is an association between the foliar spirality and the aestivation of petals. A left-handed shoot is found to produce more of right-twisting (counter-clockwise) flowers, and this situation is reversed in the case of

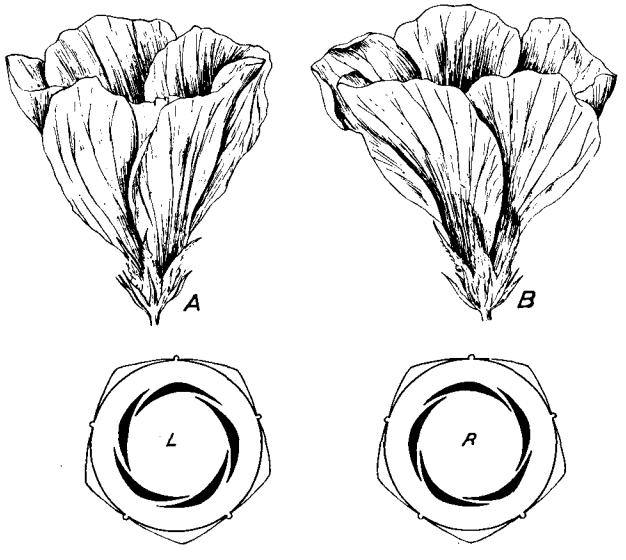
Table 1. Percentage of left- and right-twisting flowers of *Hibiscus cannabinus*

Year	Plants with left-handed foliar spiral: Plants observed	No. of flowers	Left-twisting flowers (%)	Right-twisting flowers (%)	Plants with right-handed foliar spiral: Plants observed	No. of flowers	Left-twisting flowers (%)	Right-twisting flowers (%)
1966	16	312	26.60	73.40	18	427	70.49	29.51
1967	17	401	38.65	61.35	27	686	63.56	36.44
1968	45	1225	33.31	66.69	39	1018	68.37	31.63
1969	56	1863	40.63	59.37	61	1968	61.99	38.01
1970	30	950	40.00	60.00	29	824	64.20	35.80
1971	49	2155	29.47	70.53	54	2308	68.93	31.07
1972	39	1415	42.47	57.53	41	1500	58.33	41.67
1073	38	849	34.86	65.14	40	849	62.43	37.57
1974	52	541	27.36	72.64	39	482	68.46	31.54
1975	48	995	34.37	65.63	49	1099	64.33	35.67
1976	48	775	33.03	66.97	48	886	70.20	29.80

a right-handed shoot which produces more of left-twisting flowers[6]. Observations were made at Calcutta on thousands of plants subjected to open pollination during 11 years commencing from 1966.

Each plant/shoot terminates in a long inflorescence during October–November. The flower bunch being a raceme, blooming of flowers takes place acropetally, the oldest flower occupying the base of the inflorescence. The kind of aestivation of each flower was recorded for each plant. Under Calcutta conditions, barring a few flowers at the very tip of the plant, practically all flowers set fruits. The number of fruits matured on each plant was also recorded. Dry capsules were opened, and the number of seeds per capsule was counted. Seeds of known flowers and known plants were sown year after year upto 1976.

The data on the association of foliar spiral and aestivation of corolla are presented in table 1. The left-spiralled plants produced 26–42% left-twisting flowers and 57–73% right-twisting flowers. Right-spiralled plants showed a mirror-image situation by producing 58–70% left-twisting and 29–41% right-twisting flowers respectively. Thus, there exists a distinct negative association between the foliar spiral of the plants and the kind of aestivation of corolla.



Left (a) and right-spiralled (b) flowers of *Hibiscus cannabinus* together with their partial floral diagrams.

Similar observations were made on *Abelmoschus esculentus*, *Althae rosea* and *Hibiscus radiatus*. But in none of these was the association as pronounced as in *H. cannabinus*.

In order to test whether the mean percentage of left-twisting flowers in plants with left foliar spiral is equal to the mean percentage of right-twisting flowers in plants with right-foliar spiral, the following test statistic was used:

$$T = \frac{\bar{x} - \bar{y}}{\sqrt{(1/n_1 + 1/n_2)\,\frac{(n_1-1)\,s^2x - (n_2-1)\,s^2y}{(n_1 - n_2 - 2)}}}$$

where $\bar{x}$ and $\bar{y}$ are the mean percentage of left-twisting flowers of left-spiralled plants, and the mean percentage of the right-twisting flowers in right plants respectively. s_x^2 and s_y^2 are the variances of the percentage values of the types indicated above. n_1 and n_2 denote the sample sizes for the 2 sets of data. In the present case $n_1 = n_2 = 11$. The above test statistic follows a t-distribution with d.f. n_1+n_2-2. For the present set of data

$$\bar{x} = 34.6136,\ \bar{y} = 34.4282$$
$$s_x^2 = 26.4427,\ s_y^2 = 14.1574$$

and $T = 0.0966$. This T-value is insignificant at the 5% level with d.f. 20. The above result thus indicates that the mean number of left-twisting flowers in left plants is the same as the mean number of right-twisting flowers in right plants.

In order to check up the quality of fruits produced by the plants of different spirality, the seeds were examined and the data given in table 2. From table 2 it is seen that the fruits developed from left-twisting flowers of the left-spiralled plants and the fruits developed from right-twisting flowers of right-spiralled plants were better in quality. This conclusion was derived after analyzing the

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Table 2

	Plants observed	L. fruits examined	Normal seeds	Deformed seeds	Sterile seeds	Total	R. fruits examined	Normal seeds	Deformed seeds	Sterile seeds	Total
FSL-plants											
Total	21	115	2104	181	773	3058	297	4979	410	2338	7727
Mean			18.30	1.57	6.72	26.59		16.76	1.38	7.87	26.01
FSR-plants											
Total	30	426	7192	542	2792	10526	196	3766	278	1172	5216
Mean			16.88	1.27	6.55	24.71		19.21	1.42	5.98	26.61

FSR, foliar spiral right; FSL, foliar spiral left.

data using a parallel-sample chi-square test. The comparison between the number of different types of fruits for left-twisting and right-twisting flowers of left-spiralled plants yielded a chi-square value of 26.70 (d.f. = 2) which is significant at the 5% level. Corresponding chi-square value for the other group is 35.24 (d.f. = 2), which is also significant at the 5% level. These tests thus support the conclusions drawn.

Uterine fluid from progesterone treated rabbits contains subcellular membranes

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Summary. Uterine fluid from progesterone treated rabbits was shown to be rich in subcellular membrane components consisting of vesicles and cilia-like fragments. In contrast, uterine fluid from untreated does lacked subcellular membranes. Thus, they arise when uterine sperm capacitation ability is suppressed.

Administration of progesterone to female rabbits inhibits intra-uterine sperm capacitation[2], which is an essential pre-condition for fertilization in this species and other mammals[3]. Likewise, rabbit spermatozoa fail to achieve fertilizing capacity during incubation in the progesterone-dominated uterus of a pseudopregnant doe[4,5]. The cause of this anti-fertility action by the steroid is unknown.

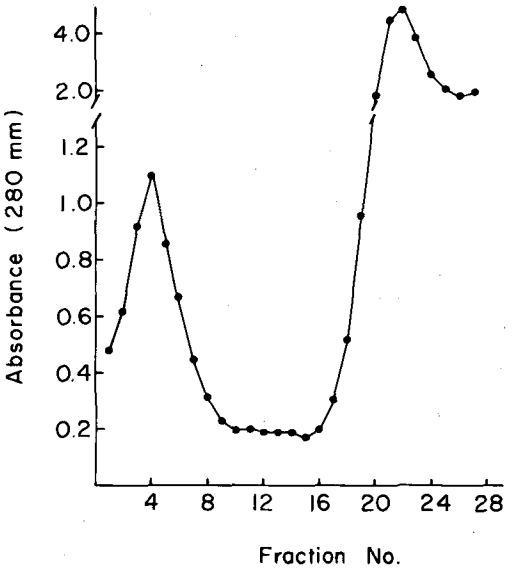

Fig. 1. Shows the OD profile at 280 nm of uterine fluid from progesterone treated rabbits after ultracentrifugation on a sucrose density gradient. The uterine fluid (6.0 ml) flushed with saline from 2 does was layered above zones of 17 ml 40 (w/v) % and 5 ml 60 (w/v) % sucrose, and centrifuged at 110,000 × g for 16 h. A sedimentable fraction, which appeared cloudy, was arrested by the 60% sucrose zone. Nonsedimentable material at the top of the gradient was clear indicating large rapidly sedimenting components had been removed.

In a series of experiments undertaken in this laboratory, it has been shown that membrane vesicles occurring in seminal plasma reversibly inhibit the fertilizing potential of uterine capacitated rabbit spermatozoa[6–9]. This communication now shows that subcellular membranes occur in rabbit uterine fluid following progesterone treatment.

Mature female rabbits, New Zealand strain, with proven fertility were obtained from a local breeder. These animals weighed from 4.0 to 5.0 kg. To facilitate collection of uterine fluid, each uterine horn was ligated at its cervical and oviductal ends. During ligation the does were anesthetized by i.v. injecting 30 mg of sodium pentobarbital/kg b.wt. Progesterone (Sigma), suspended in sesame oil, was s.c. injected at a dose of 25 mg/day for 14 days. Control animals were injected with sesame oil alone. 1 day before autopsy, the does received 75 IU of human chorionic gonadotrophin (Squibb) by i.v. injection. Each uterine horn was flushed with 3 ml of isotonic saline from a 10 ml glass syringe with a blunt No. 16 needle, which was inserted into the uterus through the cervix. The fluid obtained was centrifuged at 1000 × g for 30 min to remove any cells. The resulting supernatant was placed on a sucrose density gradient and centrifuged at 110,000 × g for 16 h in an SW27 rotor (Beckman) at 4 °C. Following centrifugation the cellulose nitrate tube containing the sucrose gradient preparation was punctured with a needle

1 The assistance of Mr R. Byrne is gratefully acknowledged. Mr K. Bedigian skilfully prepared the electron micrographs. Financial support was received from N.I.H. grant HD10206-01.

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