toriness of chronic schizophrenia to treatment with Frenquel and reserpine (5) suggests that changes may take place which are not readily reversed.

The chromatographic work does point to a physiological alteration in schizophrenia which can be controlled by means of a suitable diet. It remains for future work to establish whether such an alteration is of basic importance to the disease.

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20 December 1955

Induced Spawning in the **Indian Catfish**

In the course of an investigation of the relationship between structural changes in the pituitary and the development of ova in the common Indian catfish, Heteropneustes (Saccobranchus) fossilis (Bloch), we have attempted to induce spawning under laboratory conditions (1). This work is preliminary to a wider project designed to bring about the spawning of other major Indian carps (Labeo, Cirrhina, Catla) in confining waters. Thus restricted, either these species may become gravid but fail to ovulate, or they may discharge unfertilized eggs (2). So far as we are aware, no attempt has previously been made in India to induce spawning of food fishes by injecting fresh (or acetone dry) pituitaries of the same species. Injections of mammalian pituitary preparations result in ova that are not viable (3).

Heteropneustes breeds in large tanks just after the monsoon showers. Ghosh and Kar(4) report that the active phase of the ovary extends from late April to July, but we have collected gravid females in August and September (1955). These were taken from a local tank (Bellandur, area 893 acres) from which all of our supply comes. The occurrence of gravid fish in our collections after the normal

breeding season is doubtless due to the delay in the break of the monsoon. Weights of the gravid fish ranged from 70 to 135 g. They were held in aquaria (60 by 30 by 33 cm) that were supplied with city water from overhead cisterns, and the water was renewed daily. No additional aeration was provided, and all experiments were conducted at room temperature (24° to 25°C). The animals were not fed during the experimental period.

After a series of replications, it was found that a single injection of onequarter of a fresh pituitary from a gravid Heteropneustes homogenized in 0.5 ml of standard Holtfreter solution was quite effective.

The injections were administered intramuscularly, since it was difficult to give intraperitoneal injections without damaging the egg-laden ovisacs. Controls injected with an equal quantity of Holtfreter solution did not spawn. Only specimens weighing more than 80 g were utilized, since it was found that smaller animals did not always respond to the injections.

Because spawning usually happened during the night, we have not been able to compute the time lapse necessary for the gravid females to spawn after each injection. However, our experiments indicate that at least 12 to 14 hours must elapse before the injected females spawn in aquaria. Eggs stripped from such females the following morning were fertilized in sperm suspension in Holtfreter solution and were cultivated both in Holtfreter solution and city water, since spring water was not easily accessible to us. Development proceeded normally in both media, and hatching took place within 32 to 34 hours.

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28 December 1955

Postirradiation Protection of Rabbits by Injection of "Splenic" Plasma

Jacobson (1) has attributed the "protection" of animals by shielding of the spleen during irradiation to a "humoral factor" elaborated by the spleen. It has

| Table 1. | Comparative | data | in | series | I |
|----------|-------------|------|----|--------|---|
| and II. | | | | | |

| No. of animals | Plasma injec- tion* (cm ^s) | 30-Day sur- vival (%) | Avg. sur- vival (day) |
|-------------------|---|--------------------------------|--------------------------------|
| First seri | <i>e</i> s | | |
| 27 | 00 | 22 | 14.4 |
| 13 | 6 A | 54 | 22.2 |
| 10 | 6 | 20 | 16.0 |
| 15 | 3† | 20 | 14.4 |
| Second s | eries | | |
| 25 | | 4 | 8.9 |
| 25 | 6 A | 24 | 14.4 |
| 25 | 6 B | 18 | 12.2 |

* Intraperitoneal, except where otherwise indicated.

† Intravenous. Eight rabbits received A plasma and one B plasma. The other six had been injected before it was thought necessary to distinguish be-tween A and B plasma.

also been shown that injection of certain tissue homogenates (of spleen or bone marrow, for example) soon after irradiation is similarly protective. However, it seems that the presence of cells or cell fragments in the injected material is necessary to bring about protection. If the postulated humoral factor is elaborated by the spleen, it should be present in higher concentration in the blood leaving the spleen. Therefore, plasma of blood taken from the splenic vein should exhibit a protective action.

Such an experiment has been carried out using adult male chinchilla rabbits (2). In essence, heparinized blood was obtained from a nonirradiated donor rabbit as follows. The distal gastric coronary vein and artery were ligated at the stomach wall and then severed beyond the ligature on the spleen side. Blood from the splenic vein and artery was allowed to flow into a test tube in ice water through a polyethylene tube. This "splenic blood" is really a mixture of blood from the splenic vein, the splenic artery, and the proximal gastric coronary veins. The first 35 cm3 of blood thus collected constitutes the A sample and the second 35 cm³ the B sample. Each sample was centrifuged for 20 minutes at 2300 rev/min, and the plasma was separated.

After centrifugation of the splenic blood, approximately 18.0 cm³ of plasma was carefully removed by pipette for injections. After the three 6.0-cm³ doses of plasma had been injected, a residual drop of the plasma was placed on a slide, covered with a cover slip, and examined microscopically. No cells were found in the specimens examined. In one case, a more significant search for cells in splenic plasma was made as follows. Measured amounts of the plasma were drawn into erythrocyte- and leukocytediluting pipettes, and then the plasma

was examined on a standard blood-counting chamber. In addition, three slides were smeared with plasma, fixed with heat, and stained respectively with Wright's blood stain, methylene blue, and bromphenol blue. No cells could be found. While the possibility of the presence of some cells or cell fragments in the plasma cannot be ruled out categorically, it seems reasonable to assume that it was cell-free.

All rabbits were given, in about 10 minutes, a whole-body dose of x-rays of 1000 r measured in air at the center of the body (210 kv constant potential, 1 mm Cu half-value layer). The animal was irradiated in a lucite box 12 in. by 6 in., with the center of the body 50 cm directly below the x-ray-tube target. The plasma was injected as soon as practicable after the irradiation. In the exploratory part of the experiment, plasma was injected intravenously in some animals and intraperitoneally in others. Since intravenous injections seemed to be ineffective, they were not used in the second series. It is important to note that the rabbits were obtained in small groups as nearly identical in age and weight as possible and that a control animal in each group received x-rays only. In many cases, the animals were litter mates. The results are shown in Table 1.

Statistical analysis of the first series of 68 rabbits shows that intraperitoneal injection of A plasma gave a statistically significant (at the 0.05 level) increase in number of days surviving as compared with the controls and also as compared with the control and B-plasma animals combined. The effect of B plasma was not statistically significant. In the second series of 75 rabbits, A plasma gave a statistically significant (at the 0.01 level) increase in number of days surviving as compared with controls. All plasma A and B animals, taken together, showed a significant (at the 0.05 level) increase in number of days surviving as compared with the controls.

Thus, the protective action of freshly drawn cell-free splenic plasma (sample A) injected intraperitoneally into chinchilla rabbits soon after exposure to 1000 r of x-rays is very definite. The protec-

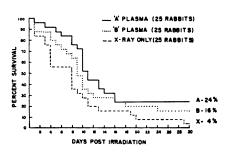


Fig. 1. Percentage survival of rabbits used in second series during 30-day test period. 15 IUNE 1956 tive action of B plasma is questionable but perhaps not zero. This indicates a greater concentration of some protective agent discharged into the splenic blood during the first part of the bleeding period. It is of interest to note in this connection that, after 10 to 25 cm³ of blood has been collected, the spleen collapses and remains quite flat thereafter.

It will be noted that the x-ray effect was greater in the second series than in the first, although the experimental procedure was the same. However, the rabbits of the first series were housed outdoors (mild weather) in the Loch Awe Rabbitry, from which all the animals were obtained, whereas those of the second series were kept in the animal care facilities of the College of Physicians and Surgeons, Columbia University. The survival curves of the 75 animals used in this series are given in Fig. 1.

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27 January 1956

Effect of Polarized Light on Polarity of Fucus

In 1941 Child (1) noted that, when Fucus furcatus zygotes were exposed to unilateral light the direction of which was reversed at hourly intervals, there arose an unspecified percentage of bipolar embryos, forms with rhizoids originating at opposite poles. I have confirmed and widely extended Child's report. In particular, it was observed that bipolar forms were effected just as frequently by oppositely directed light beams when they acted simultaneously as when they acted alternately. It was also noted that the rhizoids of the bipolar forms tended to develop perpendicularly to the light beams. However, the orientation and position of the bipolar forms in the culture vessels suggested that polarization of the light by reflection might have played a determining role.

Accordingly, I investigated the results of exposing *F. furcatus* eggs to two vertical beams of deliberately polarized light, one coming from a lamp above and the other from a mirror below the

eggs. Recently fertilized eggs were thoroughly washed with and cultured in artificial sea water (2) at 11°C in four glass petri dishes at concentrations of approximately 2 eggs/mm². One of these was cultured in the dark and three were cultured in light for 22 hours (starting at 2 hours after fertilization) and subsequently in the dark. The light source was a General Electric standard, cool, white, 20-w fluorescent lamp placed above the culture dishes. In each case, the light was collimated into a beam that was directed downward and held within $\pm 3.1^{\circ}$ of the vertical, and that was of an intensity of about 1 ft-ca. In two cases, the beam was then filtered through a Polaroid laboratory J-filter placed just above the culture vessel. In the third case, the beam entered the dish directly. A back-surfacesilvered mirror was placed just below each illuminated dish, thus reflecting upward into each a second beam of ap-

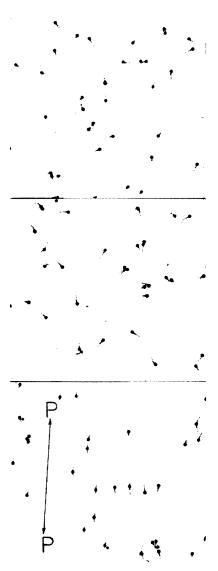


Fig. 1. Four-day-old embryos of *F. furca*tus cultured in the dark (top); in nonpolarized white light (middle); in plane polarized light (bottom). (×4).