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Effect of Drugs on Goldfish Scale Homograft Survival. (28581)

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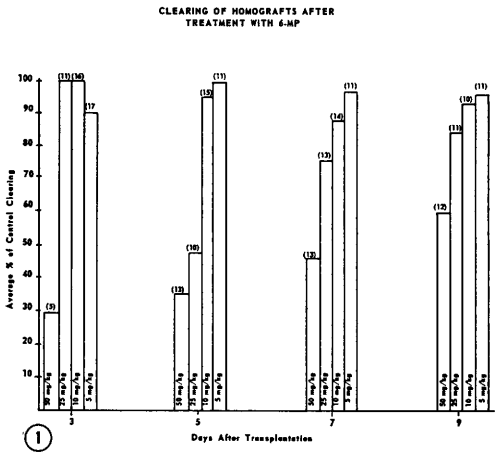
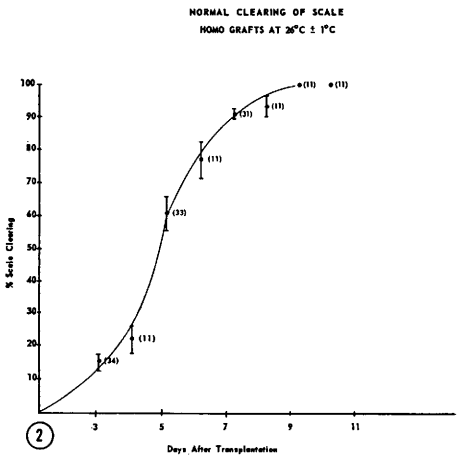
Hildemann has shown(1,2,3) that the goldfish *(Carassius auratus)* is immunologically competent and develops antibodies to scale homografts. Such scale (skin) homografts are rejected following a predictable immune response, while autografts are permanently successful and elicit little or no inflammatory reaction.

Recently there has been great interest in the effect of drugs on homograft rejection. In mammalian systems analogs of nucleic acid such as 6-mercapto purine, delay homograft rejection(4,5). Even in pure immunological systems these analogs have been shown to be effective depressants of immunological response(6,7,8). However, there have also been reports that these compounds have no effect on homograft rejection(9). On the basis of the previous findings, it was assumed that the goldfish *(Carassius auratus)* could be a useful tool in evaluating the effect of drugs on homograft reactions. This report describes some of the effects on scale homografts observed when the fish were treated with 6-mercapto purine (6-MP) and other compounds.

Method. Fish skin (scale) transplants were made according to the method of Hildemann(1). The fish were obtained locally from random bred populations at the Pacific Goldfish Farm in Westminster, Calif. All fish received an autograft as well as numerous homografts from at least 2 other fish. Drugs were given by intraperitoneal injection on different time schedules. The most frequently used procedure was injection of drug on the day of transplant and on each of 3 successive days. The transplanted scales were observed and scored on days 3, 5, 7, 9 and 11 after transplantation depending upon the treatment and rate of rejection. Observations were made using a B&L dissecting stereoscope with magnification up to 30 power. The criteria used for evaluation of scale graft acceptance were restoration and maintenance of blood flow and survival of pigment cells (xanthophores) in the scale. According to Hildemann(1) those scales which still have a substantial number of intact chromatophores are viable. The fish were kept in aquaria at 26 $\pm$ 1°C except for periods of transplantation, drug injection or observation. The fish were anesthetized with 60 mg/l of Tricaine prior to manipulation.

Results. A dose-response relationship for 6-MP is presented in Fig. 1. It can be seen that 5 mg/kg has practically no effect upon the scale chromatophore clearing or rejection and 10 mg/kg has a slight effect. Doses of 25 and 50 mg/kg definitely delay homograft rejection. The data are represented as per cent of average control scale chromatophore clearing because all scales do not clear at exactly the same rate. Each point represents the average of at least 5 scales. It should also be noted that the first observation (Fig. 1) is on the 3rd day after transplantation which is also the last day of drug injections and the treated as well as non-treated fish do not show the graded differences. Usually after the 7th day following transplantation the control scales or those scales in fish which have not received the drug are almost completely free of pigment (Fig. 2). In the fish treated with 6-MP in increasing doses, the scales are depigmented less. Blood flow was seen in





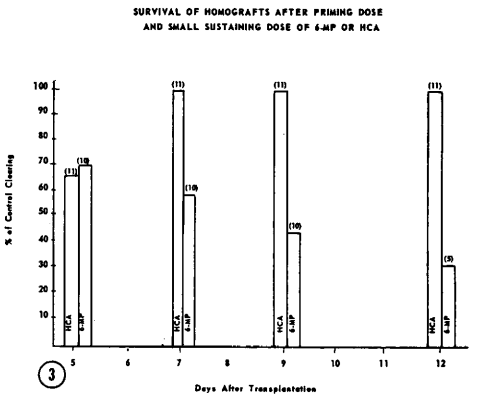


FIG. 1. Fish were injected i.p. with 6-MP dissolved in 0.05 N NaOH on day of transplant and on 3 successive days. Numbers in parentheses represent number of scales used for the average value.

FIG. 2. Dots and bars represent mean ± s.e.m. and parentheses the number of scales observed. Fish were injected on day of transplantation and on 3 successive days with 0.6% saline.

10-20% of the scales of the fish treated with 25 mg/kg or 50 mg/kg of 6-MP on the fifth day after transplantation. This was never observed in the control scales beyond the 2nd or 3rd day after transplantation. Doses of 50 mg/kg or less of 6-MP on this injection schedule were found to be non-toxic to the fish. However, higher doses usually killed the fish after 7 or more days.

A dose-dependent response was also observed in fish injected with hydrocortisone acetate (Hydrocortone, Merck) on the same injection schedule as the 6-MP. It was found that doses of less than 25 mg/kg did not markedly affect the clearing of homografts while doses of 25 mg/kg or more caused a definite delay in homograft rejection.

In exploring various dosage regimens, it was found that pretreatment one day and sometimes 2 days prior to transplantation did not alter the normal rejection pattern. Only if the drugs were injected on the day of transplantation and the days following was a marked delay in rejection time of the graft obtained. In an attempt to determine whether large doses of drug were needed to sustain the graft, the fish were given 50 mg/kg of either 6-MP or hydrocortisone acetate on the day of transplantation and then daily administration of 5 mg/kg of 6-MP or hydrocortisone acetate for 14 days.

Hydrocortisone acetate was effective in delaying the homograft reaction under these conditions but not for very long (Fig 3). However, 6-MP treatment caused a marked delay in homograft rejection and small sustaining doses of 5 mg/kg which alone were not effective (Fig. 1) prevented the homograft rejection if the animals were given a large priming dose. At this time, lower dosages for sustaining injections and lower doses for priming injections have not been investigated.

Discussion. In studies of transplantation of homografts and tumor therapy there appears to be an overlapping in the effectiveness of pharmacological agents to delay graft rejection and to slow down tumor growth (10,

FIG. 3. Fish given 50 mg/kg of drug on day of transplant and 5 mg/kg every day during experiment. Numbers in parentheses indicate number of drug treated scales used for average.

11). In addition to these observations, it has been found that metabolic antagonists such as 6-MP can inhibit general immunological responses if given during the inductive phases of the antigenic stimulation(12).

To evaluate the effects of compounds upon immunological stimuli, such as homografting in mammals, long periods are required for graft rejection to be observed. For example, Meeker *et al*(4) reported that the mean slough time in rabbits for homografts was between 17 and 26 days. Gillette, Findley, and Conway(13) found that mice normally reject homografts in 10 to 14 days. Using tumor growth as a method for evaluating anti-metabolite drugs as suggested by Humphreys *et al*(11) the median day of death obtained was between 13 and 15 days after transplantation of the tumor. The use of PAB vaccine to determine antibodies and the effect thereon of various compounds has been proposed by Berenbaum(10) as a technique for screening of agents inhibiting immune response and growth of tumors. However, this technique required 9 days to obtain the sample and then the subsequent determination of antibody titer. Sterzl(6) used 3-5 day old rabbits in evaluating the effects of drugs and found that the highest titer and most sensitive evaluation time was 5-7 days after "adoptive" transfer of spleen cells from adult donors.

The technique reported here for evaluation of anti-metabolite drugs in depressing immune responses and graft rejection has a number of advantages as a possible screening method. The technique for transplantation is simple, requires only moderate skill, physical equipment or facilities, and can be done by a technician. Sterile conditions do not have to be maintained. The fish do not require protective dressings and they can be put back into the tank immediately after transplantation. In addition, numerous transplants may be done on a single fish from multiple donors so that the opportunities for weak reactions among genetically similar fish (siblings) are minimized. Autograft controls are concomitantly performed on the same fish. At water temperature of approximately 25 to 28°C, the rejection is almost complete in about 7 days and an acceptance response or delayed rejection can be observed during this period or after this period. Goldfish are readily available, and inexpensive; therefore, many fish may be used in each experiment.

As demonstrated in the experiments reported here and previously by Goss(14) with marine teleost, fish are immunologically competent and respond in a manner quite similar to mammals to compounds which depress immunological responsiveness. The goldfish, because of his adaptability, large scales and hardiness, lends itself to this type of investigation. Preliminary observations with other purine analogs such as 6-thioguanine, and with folic acid antagonists such as A-methopterin, indicate that these compounds appear to be active in delaying the homograft reaction in the fish as they do in the mammal.

Summary. A new screening method for compounds which delay homograft rejection is described using the goldfish scale transplantation technique. It was shown that 6-Mercapto Purine and hydrocortisone acetate substantially delay homograft rejection in these fish. Moreover, sensitive dose-dependent responses were found with these drugs.

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Potentiation Between Gastrin and Histamine in Stimulation of Gastric Secretion.* (28582)

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During studies on Heidenhain pouches in dogs we noted that the responses to certain combinations of gastrin extract plus histamine were greater than would be anticipated on the basis of summation of stimulatory effects(1). The study reported here was performed to determine whether the combination of gastrin plus histamine resulted in true potentiation of stimulation of secretion of acid.

Methods. Four dogs with Heidenhain pouches were fasted overnight before each study and studies were performed no oftener than twice a week. Gastrin and histamine were given by continuous intravenous injection using a calibrated peristaltic pump that delivered 20 ml/hr. An infusion of 0.15 M NaCl at this rate was maintained throughout the study and the concentration of gastrin and histamine in the infusion fluid was varied to give the desired dosage rates. Gastrin was prepared from mucosa of the pyloric gland area of the stomach of hogs by a method previously described(1). Doses of gastrin are expressed as the wet weight of mucosa from which the extract was derived. Doses of histamine are expressed as weight of the dihydrochloride salt. Collections of gastric juice were made every 15 minutes. The volume was measured and the acidity determined by titration with 0.2 N NaOH with phenol red indicator. Output of acid is expressed as mEq/hr for the last 60 minutes of each 75-minute period.

As pointed out previously, the simple criterion that response to a combination of agents given simultaneously is greater than the sum of the responses to the agents given separately is not reliable proof of potentiation(2). We have used here the same two criteria for potentiation as were used in a previous study of cholinergic agents with gastrin and with histamine(2). Potentiation was said to exist if 1) the response to the doses of the 2 agents given together exceeded the larger of the responses to twice the dose of each agent given separately, or if 2) the response to the combined agents exceeded the maximal response attainable with either agent alone.

Results. Two plans of study were used. In Series 1 the dose of gastrin was doubled every 75 minutes through 4 steps starting with 5 g per hour. A background dose of histamine was given at a constant level throughout each experiment, beginning 2 hours before the gastrin doses were started. The doses of histamine used as a background were 0, 0.25, 0.5, 1.0, and 2.0 mg of the dihydrochloride per hour. Most of the combinations of gastrin plus histamine resulted in potentiation of stimulation as judged by criterion 1 as indicated by the encircled values ($p<0.05$) in Fig. 1. Maximal responses occurred with the intermediate doses of gastrin; the largest dose of gastrin, 40 g per hour, always gave lower secretory rates than 20 g per hour when given with any of the doses of histamine used. Since we have shown that high dosage rates of gastrin extract can profoundly inhibit histamine-stimulated secretion(1), this inflection of the dose-response curve probably represents the beginning of the reversal of effect of gastrin from a stimulatory to an inhibitory action.

In Series 2 mixtures of gastrin extract and

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