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H-Y (Male) Antigen: Detection on Eight-Cell Mouse Embryos

Abstract. *The H-Y (male) antigen is expressed on 50 percent of eight-cell embryos (2 days old) of the mouse, strain C57BL/6. This work provides additional evidence that H-Y expression is not dependent on male differentiation.*

The male-specific (H-Y) antigen (*1*) has been detected on all male tissues that have been tested in the mouse (*2*). Although some evidence suggests that H-Y expression is dependent on male hormonal differentiation, most of the data reported indicate that H-Y is expressed whenever the Y chromosome is present. Bennett *et al.* (*3*) have demonstrated that H-Y is expressed on cells of Tfm/Y mice which are lacking all testosterone-dependent characters, suggesting that the presence of H-Y is dependent on the expression of a gene or genes on the Y chromosome. Wachtel *et al.* (*4*) demonstrated that the H-Y antigen continues to be expressed on male hemopoietic cells after their transfer to female recipients, indicating that the maintenance of H-Y is independent of a male environment. By using a two-step cytotoxicity assay we have detected the H-Y antigen on eight-cell mouse embryos (day 2 of development). We thus provide additional evidence that the expression of H-Y is dependent on the presence of the Y chromosome.

We used mice of strain C57BL/6 (B6) (Jackson Laboratory). Antiserum to H-Y antigen was prepared by inoculating intraperitoneally B6 females with 25×10^6 B6 male spleen cells. The serums obtained from inoculated mice on days 7, 10, and 14 following the sixth or seventh inoculation were cytotoxic for sperm in the presence of complement. Guinea pig serum (Gibco) was used as the complement source. Our tests showed that either antiserum or guinea pig serum alone did not cause lysis of eight-cell mouse embryos. All manipulations and incubations were performed in Whitten's medium (WM) (*5*) with the following modifications: NaCl, 5.14 g/liter; sodium pyruvate (Schwarz/Mann), 0.025 g/liter; and purified bovine serum albumin (Miles Laboratories), 3.4 g/liter. To obtain eight-cell embryos, B6 females were mated with B6 males. Females were examined daily for vaginal plugs, with the morning of plug detection being defined as day 0 of pregnancy. On day 2 the pregnant females were killed by cervical dislocation. The oviducts were removed and placed in watch glasses containing WM supplemented with bovine serum albumin (WM-BSA). This and all subsequent steps were performed at 37°C. Embryos flushed from the oviducts with WM-BSA by means of a glass micropipet were collected in a small droplet (50 to 100 μl) of WM-BSA contained in a 35 by 10 mm petri dish (Falcon Plastics) coated with paraffin oil. The embryos were washed one time by transferring them to a fresh droplet of WM-BSA. The eight-cell embryos were selected and transferred to a droplet of WM containing 1 percent Pronase (Calbiochem) to partially digest the zona pellucida. Removal of the zona pellucida was achieved by repeated passage through a glass micropipet. The embryos were washed three times by successive transfer to droplets of WM-BSA, and transferred to serially diluted (in halving dilutions) antiserum to H-Y antigen in WM. The embryos were incubated in a droplet of antiserum for 30 minutes at 37°C in a humidified atmosphere of 5 percent CO_2 and 95 percent air. The embryos were then transferred to guinea pig serum (diluted 1 : 2 by volume in WM) and incubated for an additional 30 minutes under identical conditions. Embryos were scored as being H-Y^+ when one cell or more of the embryo was lysed in the presence of H-Y antibody and complement. Cell lysis was determined by light microscopy (Fig. 1).

The results in Fig. 2 indicate that 50 percent of the eight-cell embryos were affected by antiserum to H-Y antigen (diluted 1 : 4 or 1 : 8 by volume) and complement. The number of cells lysed in an affected embryo ranged from one to five.

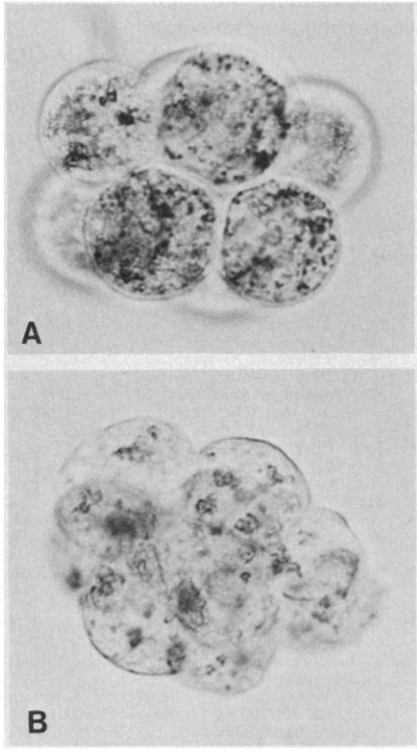

Fig. 1. Demonstration of lysis of eight-cell C57BL/6 embryos with antiserum to the H-Y antigen and complement. (A) H-Y^- embryo. (B) H-Y^+ embryo.

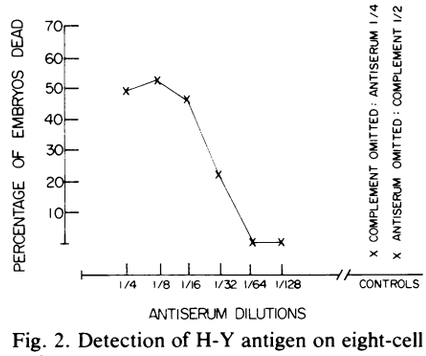

Fig. 2. Detection of H-Y antigen on eight-cell embryos.

Table 1. Specificity of embryo lysis by antiserum to H-Y antigen. Serum was used at a dilution of 1 : 8 by volume in all tests. Guinea pig serum diluted 1 : 2 by volume was used in each test as the source of complement. All tests were performed on eight-cell C57BL/6 embryos. Cytotoxic antiserum to H-Y antigen was absorbed one time with either B6 male or B6 female spleen cells and tested on eight-cell C57BL/6 embryos at a dilution of 1 : 8.

Absorption	Total number of embryos	Dead embryos	
		N	Percent
None	49	25	53
B6 male cells	30	0	0
B6 female cells	27	12	46

To confirm that the cytotoxic reaction was specific for the H-Y antigen, portions of antiserum to H-Y were absorbed with equal numbers of male and female B6 spleen cells (four volumes of serum to one volume of washed packed cells) for 30 minutes at 4°C. The absorbed serums were then tested on embryos at a dilution of 1 : 8 (Table 1). The reactivity of the antiserum to H-Y was completely lost after absorption with male cells, but the cytotoxic potential was only slightly reduced after absorption with female cells. These findings demonstrate the specificity of the antiserum for the H-Y antigen.

In addition, four-cell (1-day-old) and 10- to 16-cell (2.5- to 3-day-old) embryos were examined for the presence of H-Y. Fifty percent of the 10- to 16-cell embryos were susceptible to the cytolytic action of the H-Y antibody; however, H-Y antigen was not detectable on the four-cell embryo.

Our results support the findings of Wudl and Chapman (*6*) showing that paternal genes are expressed prior to implantation during the development of mouse embryos. Furthermore, this work provides additional evidence that the expression of H-Y antigen is dependent on the presence of the Y chromosome as suggested by Bennett *et al.* (*3*) and confirms their results that the expression of H-Y on male cells is not dependent on testosterone.

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23 April 1976

Coronary Arterial Smooth Muscle Contraction by a Substance Released from Platelets: Evidence That It Is Thromboxane A_2

Abstract. *When human platelets are aggregated by thrombin, material is released that rapidly contracts strips of spirally cut porcine coronary artery. Prevention of the contraction by indomethacin suggested mediation by a prostaglandin. The contraction produced by aggregating platelets was unlike those produced by prostaglandins E_2, $F_{2\alpha}$, G_2, or H_2, but resembled that evoked by thromboxane A_2, which is formed by platelets during aggregation.*

Evidence suggests that some cases of transient myocardial ischemia at rest result from constriction of the larger coronary arteries (*1*). An acute reduction in flow resulting from coronary constriction could also conceivably set the stage for myocardial infarction or fatal cardiac arrhythmias.

The intimate involvement of platelets in the development of atherosclerotic lesions (*2*) led us to investigate the possibility that platelet aggregation could interrupt coronary flow by releasing a substance that constricts the major coronary arteries.

To investigate the possible release of vasoconstrictors from platelets, we placed suspensions of washed human platelets that had been stimulated to aggregate into baths containing isolated coronary arteries. Washed platelets were prepared from blood of normal male volunteers who had not taken aspirin for at least 1 week (*3*). The left coronary artery was removed from fresh porcine hearts, spirally cut into strips and placed in Krebs-Ringer bicarbonate buffer (37°C) saturated with 95 percent O_2 and 5 percent CO_2 and containing 8 μM indomethacin. After 3 hours of equilibration, changes in isometric tension were measured in arterial strips that were under tension (1.25 to 1.50 g). Contractile responses were expressed relative to the maximum tension developed with 85 nM prostaglandin E_2 (PGE_2). Washed platelets were incubated in a Payton aggregation module and then stimulated to aggregate with thrombin (1 unit per milliliter of washed platelets). At various times after the addition of thrombin, 0.75 ml of the platelet suspension was removed from the aggregation module, quickly diluted to 3 ml with bathing medium and transferred to 3-ml coronary artery baths. In some experiments, the synthesis of prostaglandins was inhibited by adding indomethacin (27 μM) (Fig. 1) to the platelets before thrombin stimulation. Because aggregating platelets also release 5-hydroxytryptamine (5-HT), the artery strips were first treated with the 5-HT antagonist, dihydroergotamine (0.7 μM), except where indicated otherwise.

After the addition of thrombin to the platelets, maximum amounts of a contractile substance existed within 15 seconds (Fig. 2). The contraction onset was rapid and reached a maximum in 1.1 ± 0.1 minutes (mean ± standard error; N = 8) as shown in Fig. 3A. Two minutes after thrombin was added to the platelets, the evoked coronary contraction was smaller, and by 4 to 8 minutes no contraction was induced. These data suggested that a labile contracting substance was released by the platelets in the presence of thrombin. Indomethacin, which inhibits prostaglandin formation but not prostaglandin action, prevented contraction, suggesting that the contractile substance was a prostaglandin. The contractions produced by 255 nM PGE_2 (Fig. 3B) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were, in contrast to those produced by thrombin-stimulated platelets, slow to develop and persisted long after 4 minutes. Prior treatment of the strips with dihydroergotamine unmasked an unidentified relaxing factor which lowered the baseline tension but did not block the contraction produced by thrombin-induced aggregation.

The prostaglandin cyclic endoperoxides G_2 and H_2 (PGG_2, PGH_2, Fig. 1) contract isolated rabbit aortic smooth muscle (*3, 4*). To test the effects of these cyclic endoperoxides on coronary smooth muscle, PGG_2 and PGH_2 were synthesized from arachidonic acid by using the cyclooxygenase from sheep seminal vesicular microsomes (*3*). After they were extracted from the incubation mixture, PGG_2 and PGH_2 were separated by silicic acid chromatography (*3*). Twenty

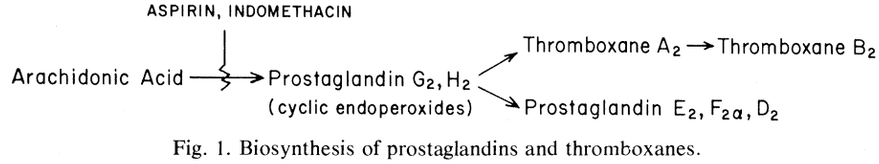

Fig. 1. Biosynthesis of prostaglandins and thromboxanes.