# Acquired Immunological Tolerance of Male Skin Isografts in Adult Female Mice Injected with Whole Blood.* (26507)

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Lasting immunological tolerance of skin homografts in animals injected with whole blood from the prospective donors has been obtained during fetal life in chicks(1) or during the neonatal period in chicks(2), rats(3) and dogs(4). More recently Billingham and Silvers(5) have obtained permanent tolerance of male skin isografts in female mice of the C57 Bl/6 strain injected intraperitoneally at birth with various concentrations of peripheral blood leucocytes taken from male donors. However, attempts to establish tolerance of homologous skin grafts in weanling and adult rabbits or rats by injecting these animals with whole blood taken from the prospective donors have resulted only in a significant delay in survival time of subsequent homologous grafts(6,7).

On the basis of these results and certain theoretical considerations we have decided to investigate further the possibility of inducing permanent tolerance of male skin isograft in adult female mice of the C57Bl/1 strain previously transfused with different amounts of whole blood taken from mature male donors of the same strain. Female mice of this strain regularly reject skin grafts taken from isologous males although the weak histocompatibility difference between donor male and recipient female allows induction of tolerance in the females to the male graft by exposure to male antigens in the form of viable spleen cells during the neonatal period as well as in adult life(8).

The experiments reported herein demonstrate that lasting tolerance of male skin isografts can, indeed, be induced in adult female mice of the C57Bl/1 strain by pretreatment of these animals with whole blood taken from isologous mature male donors.

*Method.* Adult male and female mice of the C57Bl/1 strain were used. Pooled whole blood obtained by decapitation under ether anesthesia of stock C57Bl/1 adult male mice was collected in a test tube containing a minimal amount of heparin solution.§ This blood was immediately injected intravenously into the lateral subcutaneous vein of the tail of several groups of isologous females 60 days of age. Recipient mice were divided into groups according to the amount of blood transfused, as follows: 0.1 cc, 0.25 cc, 0.50 cc, 0.75 cc and 1 cc of whole heparinized blood. Control groups consisting of untreated 60 day old C57Bl/1 female mice and mice receiving 0.75 cc of isologous female whole blood were also prepared.

Three to 4 days after blood transfusion, pretreated and untreated female mice received a full-thickness abdominal skin graft taken from male donors of the same strain. The technic of skin grafting as well as the criteria used to determine the success or failure of the graft was the same as that previously described(9).

Mice were housed individually in plastic cages and fed Purina Laboratory Chow and tap water. Grafted animals were observed for at least 5 months and only those animals showing a well established graft growing copious hair in a direction opposite to that on the skin of the host were judged to be tolerant of male skin isograft.

*Results.* The results are summarized in Table I. Of the group of untreated C57Bl/1 female mice grafted with male skin 4 out of 19 (21%) accepted this graft. Likewise, of the control group consisting of C57Bl/1 female mice injected with 0.75 cc of isologous female whole blood 2 out of 12 (17%) accepted the male skin isograft. On the other hand, of the females pretreated with isologous whole blood taken from male donors each of

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§ Liquaemin Sodium, Organon, Inc.

TABLE I. Induction of Lasting Immunological Tolerance of Male Skin Isograft in Adult Female Mice of C57Bl/1 Strain Injected Intravenously with Various Amounts of Male Whole Blood.

| Host strain | Skin donor strain | Pretreatment, whole blood cc/mouse | Sex | No. females accepting male skin isografts/ No. animals grafted |
|---|---|---|---|---|
| C57Bl/1 ♀ | C57Bl/1 ♂ | .10 | ♂ | 3/15 |
| *Idem* | *Idem* | .25 | ♂ | 5/10 |
| " | " | .50 | ♂ | 12/14 |
| " | " | .75 | ♂ | 10/12 |
| " | " | 1.00 | ♂ | 5/7 |
| " | " | .75 | ♀ | 2/12 |
| " | " | None | | 4/19 |

the groups except that in which 0.1 cc of blood was injected showed a significant increase in male skin isograft acceptance as compared to that observed in groups of control animals. In the group injected with 0.10 cc 3 out of 15 treated females accepted skin isografts while in the 0.25 cc, 0.50 cc, 0.75 cc and 1.00 cc treated groups respectively 5 of 10, 12 of 14, 10 of 12 and 5 of 7 females accepted male skin isografts.

*Discussion.* These results are of interest for several reasons. First, they confirm our previous studies in demonstrating that acquired immunological tolerance can be induced in adult female mice of the C57Bl/1 strain by pretreating these animals with male spleen cells injected intravenously in large number or by placing the females in celomic parabiosis with isologous males(8). In the experiments reported herein tolerance of male skin isograft was obtained in adult females injected intravenously with various amounts of peripheral blood taken from isologous male donors. However, establishment of tolerance by this method appears to be related to the amount of male blood injected. For example, while 0.10 cc of fresh whole blood was not sufficient to bring about immunological tolerance of male skin isograft, doses ranging from 0.25 to 1.00 cc induced permanent tolerance in many instances. The difference in incidence of male skin acceptance observed between controls and all experimental groups injected with at least 0.25 cc of blood is statistically significant at the 1% level. The fact that establishment of tolerance by injecting whole blood is related to the dose of blood administered suggests that a minimum amount of blood must be used to produce tolerance, beyond which greater amounts have no appreciable advantage.

Second, our results demonstrate the possibility of inducing immunological tolerance in adult individuals using peripheral blood cells as the preconditioning material. Billingham *et al.*(2) have demonstrated that while whole blood was successful to a small degree as pretreating material of fetal mice to induce this phenomenon, lymphoid tissue was far more efficacious. However, this advantage of lymphoid tissue over whole blood was not observed by Puza and Gombos(4) who gave whole blood to neonatal dogs by the intravenous route.

One question explicit in these results is: Which peripheral blood cells are responsible for establishing immunological tolerance? The smallest dose of whole blood required to produce satisfactory degree of tolerance was 0.25 cc which represents approximately 1,500,000 viable white blood cells. This is less than the number of spleen cells necessary for induction of tolerance in adult mice (8). Yet many of the peripheral cells are end stage elements considered incapable of replication. It could be that the red blood cells themselves play some assistive role in inducing in the recipient animal a state similar to that of immunological paralysis(10) thus allowing time to the injected cells to establish themselves and perhaps replicate to reach a sufficient number to abolish the homograft reaction. We are now investigating this hypothesis.

It seems clear that immunological tolerance of male skin isografts can be produced in adult female mice of the C57Bl/1 strain by pretreatment of these mice intravenously with various amounts of fresh whole blood taken from mature isologous male donors.

*Summary.* Permanent acquired immunological tolerance has been established in adult C57Bl/1 female mice to isologous male skin graft using pooled whole blood obtained from C57Bl/1 males as a pre-conditioning material. Tolerance using this technic is a dose related

phenomenon. Doses of 0.25 cc of whole blood or greater produce a long lasting tolerance.

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## Effect of Reserpine on Peptic Ulceration and Gastric Blood Flow in Dogs.* (26508)

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The clinical use of rauwolfia alkaloids has found wide acceptance since their recent introduction to this country mainly for treatment of hypertension and certain anxiety states. However, as with many drugs, these compounds produce undesirable side effects on other physiological systems. Duodenal ulcer, hemorrhage, and perforation have been reported in patients treated with reserpine(1,2). It is not known whether patients treated with rauwolfia drugs develop gastric complications as a result of therapy, because of their disease process and emotional constitution, or a combination of these factors. We attempt in this study to elucidate some of the effects of reserpine on gastric physiology and peptic ulceration in experimental animals.

Peptic ulceration does not occur spontaneously in either dogs or cats. However, ulceration can be produced in laboratory animals by administration of histamine in beeswax(3). This method has proven useful in evaluation of surgical procedures and medications on peptic ulcer diathesis.

The first part of this study demonstrates the effect of reserpine on histamine-induced ulceration in dogs and cats. The second portion shows the effect of reserpine on gastric blood flow.

*Method.* A. *Ulcer provocation.* Adult mongrel dogs and cats were used in these experiments. The histamine in beeswax mixture was prepared as described by Code and Varco(3). The dogs were kept in separate cages and the cats were kept 4 to a cage. All animals received food and water *ad libitum.* Although the exact amount of food ingested was not measured, the animals receiving reserpine consumed less of the rations offered.

The animals were divided into 6 groups. Group I (9 dogs) received 30 mg of histamine in beeswax I.M. daily. Group II, (8 dogs) received 1 mg of reserpine I.M. in addition to the histamine. Group III, (8 dogs) received only 1 mg reserpine daily. Group IV (8 cats) received 15 mg histamine in beeswax daily. Group V (8 cats) received 0.5 mg of reserpine in addition to the histamine. Group VI (11 cats) received 0.5 mg reserpine only.

Each animal was injected with histamine in beeswax and/or reserpine intramuscularly at the same time each day. The dosage of reserpine used caused miosis, diarrhea, and lethargy in both dogs and cats. At time of death or sacrifice, the stomach and duodenum of all animals were examined grossly for erosion or ulceration. For the purpose of this

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