sented. As may be noted, ventricular ratios of the metacorticoid rats receiving acetazolamide-reserpine co-treatment were significantly less than ratios of the control hypertensive rats. Analysis of the data by means of Dunnett's multiple comparison procedure (7) revealed that all of the mean ventricular ratios of the treated rats less than 3.84 are significantly smaller than the mean of 4.23 for the untreated hypertensive control rats.

Discussion. The absence of a significant reduction in arterial blood pressure following repetitive administrations of chlorothiazide to hypertensive rats attests to the difficulty encountered experimentally in demonstrating an antihypertensive action for this type of compound. The summary table of Sturtevant(8) indicating that chlorothiazide lowers the blood pressure of metacorticoid rats cannot be assessed since supporting data were not presented. The augmented antihypertensive action of reserpine-chlorothiazide co-treatment over that produced by either drug alone is in accord with the usual clinical finding(1,2). The pronounced antihypertensive action of acetazolamide-reserpine combination treatment was striking. The sustained hypotension encountered following repetitive co-treatments was of a greater magnitude than that previously recorded under identical experimental conditions after repetitive administrations of ganglioplegic and vasodilator agents. The interesting finding that acetazolamide-reserpine co-treatment tended to obviate development of left ventricular cardiac hypertrophy indicates that the combination therapy exerted a desirable effect on both the hemodynamic and the morphologic disturbances associated with metacorticoid hypertension.

Summary. An increased antihypertensive action of a combination of reserpine and acetazolamide over that produced singly by either drug was demonstrated in metacorticoid hypertensive rats. Acetazolamide alone had a moderate antihypertensive action, and the enhanced reduction in blood pressure following co-treatment was an additive rather than a potentiated effect. The typical hypertensive left ventricular cardiac hypertrophy was significantly reduced in the metacorticoid rats receiving acetazolamide-reserpine.

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Tolerance of Skin Homografts Induced in Adult Mice by Multiple Injections of Homologous Spleen Cells.* (26373)

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It seems now well established that acquired immunological tolerance of tissue homografts can be induced in adult mice of certain strains closely related genetically. For example, tolerance of male skin isografts was obtained in adult female mice of C57Bl (Subline 1) and A strains by a single intravenous injection of

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approximately 20 million spleen cells taken from male donors, or by placing the females in celomic parabiosis with isologous males(1). Similarly, adult Z (C3H) strain mice less than 1 month of age were made tolerant of (Z × Ce)F_1 hybrid skin homografts by intravenous injection of viable spleen cells taken from donors of the (Z × Ce) F_1 hybrid cross(2). Finally, mice of the Z (C3H) strain were made tolerant of either (A × Z) F_1 hybrids or Ce strain mice when animals of these strains were joined in celomic parabiosis (3). In the case of parabiosis it was also demonstrated that the time during which parabiosis had to be maintained to induce lasting tolerance varied with the strains of mice in parabiosis and apparently with degree of histocompatibility difference between the 2 strains(4). Thus, while only 4 days of parabiotic union between female and male mice of either A or C57Bl (Subline 1) strain were sufficient to abrogate immunological rejection reaction in the females to skin grafts taken from the male partner, 30 to 45 days days of parabiosis between Z(C3H) and homologous (A × Z) F_1 hybrid mice were necessary to permit establishment of tolerance of (A × Z) F_1 hybrid skin homograft in the Z (C3H) strain parabionts. Similar findings were obtained in case of parabiosis between Z (C3H) and Ce animals, although in this instance while only 27 days of parabiosis were necessary to produce tolerance of Ce skin homografts in the Z (C3H) partners, 64 days were necessary to induce tolerance of Ce skin in the Z (C3H) strain parabiont.

We interpreted these results to indicate that tolerance thus produced resulted from a continuous exchange of transplantation antigens in form of intact cells between both members of the pair in such amounts as to inhibit the homograft reaction. This state of nonreactivity could then permit establishment of cells capable of replication in the respective hosts, and these by producing antigen would be sufficient to maintain the tolerant state. On the basis of these observations it was postulated that were it technically possible to inject large enough numbers of viable cells intravenously with sufficient frequency over a sufficiently long period it might be possible to produce lasting tolerance by injection of homologous adult cells in adult recipients.

In the experiments reported here attempts have been made to simulate the parabiosis phenomenon and induce tolerance in mice subjected to repeated injections of large numbers of homologous viable spleen cells. The results can be interpreted as substantial support for our hypothesis.

Method. Mice of the Z (C3H) strain and (A × Z) F_1 hybrids of both sexes were used. Two-month-old mice of the Z (C3H) strain were repeatedly injected with viable spleen cells from homologous donors of the (A × Z) F_1 hybrid cross either by the intraperitoneal route alone, or alternatively by the intravenous route and the intraperitoneal route. The method of preparation of the spleen cells was as previously described(5). Mice to be injected were divided into groups according to number of spleen cells injected, duration of treatment and route of administration (Table I). Group I received a total of approximately 200 million viable spleen cells taken from donors of the (A × Z) F_1 hybrid cross administered in 4 intraperitoneal injections during 1 week. Group II received a total of approximately 1 billion 300 million cells of the same hybrid strain in 3 weekly intraperitoneal injections during 4 to 6 weeks. Group III received a total of approximately 500 million spleen cells in 3 weekly intraperitoneal injections over a period of 7 to 11 weeks. Group IV received a total of 1½ billion spleen cells injected over a period of 7 weeks. In this instance injections were performed daily during the first 2 weeks alternating the intravenous and intraperitoneal route and twice weekly during 5 consecutive weeks. Group V was not treated and remained as controls.

One day after the last injection experimental as well as control animals received a full-thickness abdominal skin homograft taken from (A × Z) F_1 hybrid donors. All donor and recipient mice were of the same sex. The technic of skin transplantation was the same as previously described(5) consist-

TABLE I. Induction of Immunological Tolerance in Z (C3H) Strain Mice Repeatedly Injected with Viable Spleen Cells Taken from (A × Z) F_1 Donors.

Group	Total No. of spleen cells inj. (millions)	Duration of treatment (wk)	No. of Z (C3H) mice accepting (A × Z) F_1 hybrid skin homograft/No. of mice grafted
I	200*	1	0/22
II	1,300†	4–6	2/22
III	500†	7–11	1/31
IV	1,500‡	7	6/7
V	none	—	0/42

* 4 injections intraper.
† 3 intraper. inj./wk.
‡ Intraper. and intrav. (for schedule of treatment, see text).

ing essentially in transfer of a full-thickness abdominal piece of skin measuring 2 × 2 cm from the donor to the back of the recipient. The graft was turned 180° to facilitate determination of subsequent success or failure of the graft. In such grafts hair on successful grafts grows in a direction opposite to that on the skin of the host. Grafts were inspected 3 times a week for not less than 4 months before a final determination of success or failure of the graft.

After operation grafted mice were housed individually in plastic cages and fed Purina Laboratory Chow and tap water.

Results. The results are recorded in Table I. Mice of Group I rejected the homologous skin grafts in all instances. However, in mice of Group II pretreated with 1 billion 300 million homologous spleen cells intraperitoneally over a period of 4 to 6 weeks 2 of 22 (9%) accepted the skin grafts taken from (A × Z) F_1 hybrid donors. Only 1 of 31 mice of Group III receiving intraperitoneal injections of approximately 500 million homologous spleen cells over a longer period of time (7 to 11 weeks) accepted the F_1 hybrid skin homograft. Finally, in Group IV in which administration of cells was carried out by both intraperitoneal and intravenous route, 6 out of 7 mice successfully accepted the (A × Z) F_1 hybrid homologous skin graft (Fig. 1). It was interesting to note that the one mouse rejecting the graft in this group was able to retain it for approximately 2 months.

As expected, all Z (C3H) control mice grafted with homologous (A × Z) F_1 hybrid skin rejected this graft in 12 to 15 days after operation (Group V).

Discussion. These results clearly demonstrate that immunological tolerance of homologous skin grafts can be induced in adult inbred mice subjected to repeated administration of viable homologous spleen cells. In our experiments adult Z (C3H) strain mice were used as recipients and F_1 hybrids resulting from cross between A strain females and Z (C3H) males were used as homologous donors of the spleen cells.

This confirms and extends previous reports from this laboratory demonstrating that tolerance of (A × Z) F_1 skin homografts could be established in adult Z (C3H) mice after placing in celomic parabiosis animals of these 2 homologous strains (3). In the latter instance, the essential duration of the parabiotic state to overcome the immunological capacity of the Z (C3H) partner against the (A × Z) F_1 hybrid was approximately 30 days, when permanent tolerance of the F_1 hybrid skin was established.

In the experiments reported here the parabiotic phenomenon was simulated by repeated administration of spleen cells taken from (A × Z) F_1 hybrid donors into adult recipients of the Z (C3H) parent strain. Administration of homologous cells was carried out over periods of one to 11 weeks. The results indicate that a high incidence of tolerance is obtained when cells are administered by both intraperitoneal and intrave-

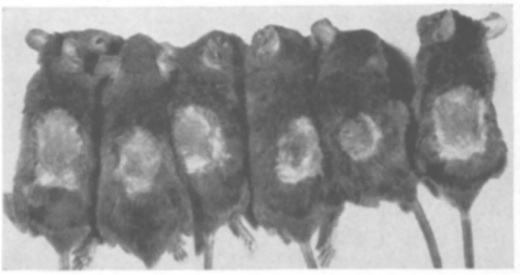

FIG. 1. Z (C3H) strain mice bearing successful homologous skin grafts taken from (A × Z) F_1 hybrid donors. Tolerance induced by repeated intrav. and intraper. administration of (A × Z) F_1 hybrid spleen cells over a period of 7 wk. Picture taken 4½ mo after skin grafting.

nous routes since 6 out of 7 animals thus treated became tolerant and accepted skin grafts taken from homologous donors of the (A × Z) F_1 cross. However, when spleen cells were injected by the intraperitoneal route alone this treatment was not quite sufficient to induce permanent tolerance since only 3 out of a total of 75 Z (C3H) strain animals accepted the F_1 hybrid skin. Even here, however, a suggestion that the immunological barrier was bulged is indicated since none of the non-treated controls accepted F_1 hybrid homologous skin grafts.

Interpretation of these results, as in the case of our studies on tolerance induced by parabiosis, is difficult in the light of current theories regarding immunological tolerance and homotransplantation. In the case of parabiosis we have previously postulated that tolerance of skin homografts induced by this method might be the result of a continuous exchange of all transplantation antigens in the form of intact cells being transferred from one partner to the other in such amount as to inhibit capacity for a homograft reaction. If the parabiosis is maintained for a sufficient length of time this state of nonreactivity could permit establishment of the homologous cells in a form capable of replication in the host thereby maintaining the tolerant state.

By repeatedly injecting homologous spleen cells into adult recipients in a manner designed to simulate as nearly as possible the parabiotic state, tolerance has been obtained in adult animals. The fact that large numbers of spleen cells had to be given during a relatively prolonged period of time combining the intravenous and the intraperitoneal route to achieve tolerance seems to favor this interpretation.

Whatever the final explanation of these findings, it seems clear that immunological tolerance of skin homografts can be induced in adult mice of certain strains submitted to repeated administration of homologous viable spleen cells. It also seems clear that dosage of cells injected, length of time during which treatment has to be maintained and route of administration of cells are also important factors in induction of this phenomenon. These factors, in turn, as in the case of tolerance induced by parabiosis, might also be a function of the antigenic disparity between host and donor.

Summary. 1. Adult mice of the Z (C3H) strain have been made tolerant of skin homografts of the (A × Z) F_1 hybrid strain by repeated intravenous and intraperitoneal injections of homologous spleen cells. 2. Repeated intraperitoneal injections of homologous spleen cells generally failed to induce a tolerant state, although 3/75 recipients accepted the homologous skin homotransplant.

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