tween the dietary groups. The finding that the feeding of a 50-50 mixture of glucose and galactose resulted in a higher rate of glucose uptake indicates that the effect of lactose involves metabolic effects other than those of the absorbed monosaccharides.

Previous studies have shown that the *in* vitro rate of glucose uptake by liver tissue (6) and liver enzyme activities(7) varies after feeding different carbohydrates. The present results show that the glucose metabolizing activity of adipose tissue was also influenced by the carbohydrate of the diet.

The differences in glucose uptake in the *ad libitum* fed groups were directly related to weights of the epididymal fat bodies or estimated total fat content of the carcass. This relationship is in agreement with the finding that rate of triglyceride synthesis is directly related to rate of glucose metabolism by adipose tissue(2).

Summary. Comparisons were made of the *in vitro* rate of glucose uptake by the epididymal fat pads from rats fed lactose, glucose or a 50-50 mixture of glucose and galactose. Glucose uptake of insulin-stimulated adipose tissue from rats fed lactose was significantly lower than in the other dietary groups. A direct relationship was observed between rate of glucose uptake and weight of the epididymal fat pads or estimated carcass fat content.

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Transfer of Tolerance Induced by Parabiosis to Isologous Newborn Mice.* (26686)

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Tolerance of skin homografts has been induced in adult inbred mice by establishing celomic parabiosis between members of various homologous strains. For example, adult mice of the Z§ strain joined to homologous (A × Z) F_1 hybrids or to mice of the Ce strain resulted in tolerance whereby the Z partner accepted skin homografts taken from either (A × Z) F_1 hybrid cross or Ce strain donors(1). Further experimentation demonstrated that the length of time during which parabiosis had to be maintained to achieve tolerance varied according to the strains of mice joined(2). Induction of tolerance in adult mice by this procedure was interpreted as being the result of a continuous exchange of transplantation antigens in the form of intact cells between members of the pair resulting in a suppression of the homograft reaction thus permitting establishment in the homologous host of replicating cells containing the corresponding transplantation antigens(3,4).

On the other hand, our previous reports have indicated that immunological tolerance of tissue homografts induced by antigenic exposure during the neonatal period can be transferred to isologous mice by injecting these animals at birth with viable spleen cell

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§ Z is used to designate mice of the C3H strain originally obtained from Dr. J. J. Bittner's mouse colony.

TABLE I. Transfer of Tolerance Induced by Parabiosis to Isologous Newborn Mice.

Spleen donor strain*	Host strain	No. of mice accepting AZ F_1 homograft/No. of animals grafted	%
Z tol. of AZ F_1 (parabiosis 30 days)†	Z	0/20	(0)
Idem (parabiosis 150 days)‡	"	19/28	(68)
None	"	0/24	(0)

* The dose of spleen cells inj. intrav. into newborn Z strain mice was 4-5 million cells/mouse.
† Tolerance induced by Z—AZ F_1 parabiosis maintained during 30 days.
‡ Tolerance induced by Z—AZ F_1 parabiosis maintained during 150 days.

suspensions taken from adult tolerant donors. In other words, tolerance of "B" strain tissue induced at birth in "A" strain mice can be transferred to isologous "A" strain animals by injecting these mice at birth with spleen cells taken from "A" tolerant of "B" adult donors(5). This was interpreted as an indication that in the tolerant host all of the transplantation antigens presumably in the form of many intact cells derived from the original donor are present in the reticulo-endothelial system of the tolerant individual.

In view of these results it was considered important to ascertain whether or not tolerance of tissue homografts induced in adult mice by means of parabiosis could also be transferred to isologous animals by injecting them at birth with viable spleen cells taken from tolerant donors. It was reasoned that if tolerance induced by parabiosis could indeed be transferred in this fashion it would be a strong indication that the parabiosis-induced tolerance in adult mice as well as that obtained in neonates may be the result of persistence of homologous cells in the reticulo-endothelial system. The results reported herein seem to support this concept.

Method. Adult mice of the Z strain and F_1 hybrids resulting from the cross between A strain females and Z males were used. When mice were approximately 3 months of age a "Z—AZ F_1" celomic parabiosis was performed by the technic previously described(3). Parabiotic pairs were housed in plastic cages containing one pair per cage and fed laboratory chow and tap water. At various time intervals, namely 30 and 150 days following establishment of parabiosis groups of parabiotic pairs were surgically separated and 8 days later the Z strain parabiont received a full-thickness abdominal skin homograft taken from donors of the AZ F_1 hybrid cross. The technic of skin grafting was the same as previously described (6). Mice accepting the skin homografts for a period of time of at least 3 months were considered as tolerant and submitted to removal of their spleens to prepare a spleen cell suspension in Ringer-Locke's saline solution containing approximately 10 million viable spleen cells per 0.1 cc. This suspension was immediately injected intravenously into newborn mice of the isologous Z strain in a dose of 0.05 cc per mouse. The technic for preparation of the spleen cell suspension was described previously(6) and the method of intravenous injection in newborn mice was the same as that recommended by Billingham and Brent(7).

Injected mice were raised by their own mothers and at approximately 2 months of age received a full-thickness abdominal skin homograft taken from adult AZ F_1 hybrid donors. After skin grafting, mice were singly housed in plastic cages and fed laboratory chow and tap water. Grafts were inspected daily and success or failure scored at the end of the 5th month following transplantation.

Results. Results are listed in Table I and Fig. 1. Of the group of 20 animals injected intravenously at birth with viable spleen cells taken from Z strain donors made previously tolerant of AZ F_1 hybrid tissue by virtue of parabiosis of 30 days duration, none were tolerant and rejected the AZ F_1 hybrid skin homografts. In contradistinction, 19 of 28 mice similarly injected at birth with spleen cells derived from Z mice made tolerant of AZ F_1 hybrid by "Z—AZ F_1" parabiosis maintained for 150 days became tolerant and successfully accepted AZ F_1 homologous skin grafts. As expected, none of the non-injected

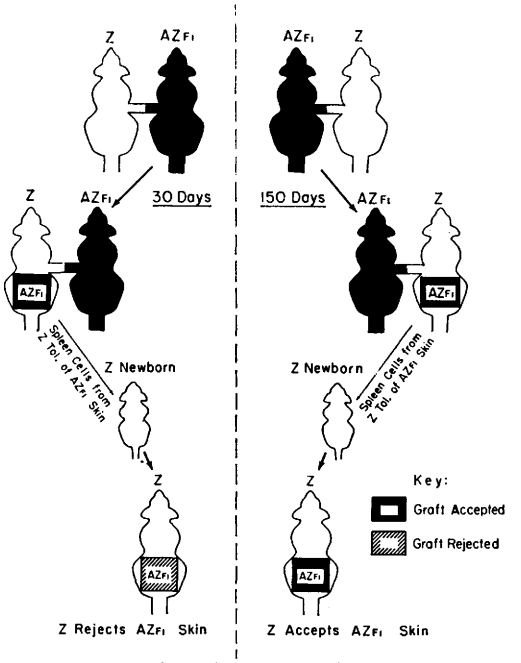

FIG. 1. Transfer of tolerance induced by parabiosis. Spleen cells taken from mice of Z (C_3H) strain made tolerant of AZ F_1 hybrid tissue by virtue of Z (C_3H)—AZ F_1 parabiosis of 150 days duration are able to induce tolerance of F_1 strain when inj. into newborn mice of the Z (C_3H) strain. However, if inj. spleen cells are taken from Z (C_3H) donors tolerant of AZ F_1 hybrid tissue as a result of parabiosis of 30 days duration, tolerance of AZ F_1 tissue could not be obtained.

controls accepted the homologous F_1 hybrid skin transplant.

Discussion. The results are of interest from several points of view. They confirm our previous reports that tolerance of homologous tissue transplants can be induced in adult mice by establishing a celomic parabiosis between members of certain homologous strains(1,2,3). The mechanism whereby tolerance is achieved by this method was postulated to be the result of a continuous exchange between both parabionts of all the transplantation antigens in the form of intact cells. If this exchange is maintained for a sufficient length of time permanent establishment of these cells in the homologous host occurs and lasting immunological tolerance is the rule. The results herein reported also demonstrate that tolerance of AZ F_1 skin homograft induced in adult Z mice by the parabiosis procedure can be transferred to isologous Z strain mice by intravenously injecting these animals at birth with spleen cells taken from the Z tolerant of AZ F_1 hybrid donors. It is interesting to note that tolerance can be transferred only when the spleen cells were derived from mice made tolerant by virtue of Z—AZ F_1 parabiosis of 150 days duration and not at all when these cells were taken from tolerant animals resulting from parabiosis of only 1 month duration. Transfer of tolerance to isologous mice by injection of spleen cells during the neonatal period can be interpreted as being the result of transfer of homologous AZ F_1 cells in residence in the reticulo-endothelial system of the chimeric tolerant Z donor resulting from the parabiotic exchange of cells. The fact that transfer of tolerance was successfully achieved when the donors of the spleen cells had been maintained in parabiosis for 150 days and failed when the spleen cells were derived from similar donors maintained in parabiosis for only 30 days can be interpreted as a function of the actual number of homologous cells in residence in the reticulo-endothelial system of the donor animal at the time the transfer of tolerance was attempted. In this regard it is feasible that the number of homologous cells present in the spleen of the tolerant donor after 30 days of parabiosis, although enough to maintain the state of non-reactivity, was not quite sufficient to induce tolerance after transferring these cells to isologous newborn mice. In contradistinction, the spleen derived from tolerant donors by virtue of parabiosis of 150 days duration might contain many more homologous cells, in sufficient number to induce tolerance when transferred to isologous newborn individuals.

It seems clear, therefore, that tolerance of homologous tissue grafts induced by the parabiosis procedure in adult mice of certain donor-host strain combinations results from an exchange of cells containing all the transplantation antigens establishing residence and almost certainly replicating themselves in the reticulo-endothelial system of the homologous host. The transfer of this form of tolerance by injecting isologous newborn animals with spleen cells derived from tolerant donors supports this interpretation.

Summary. Immunological tolerance of AZ F_1 hybrid tissue homograft induced in adult Z mice by virtue of parabiosis of 150 days duration can be transferred to isologous Z strain mice by injecting these animals at birth with spleen cells taken from Z donors tolerant of AZ F_1 tissue. Tolerance resulting from parabiosis of only 30 days duration failed to be transferred by the same procedure.

In the light of these results the mechanism of tolerance induced in adult individuals by the parabiosis procedure is discussed.

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Effect of Hydrocortisone on Human Cells in Tissue Culture.* (26687)

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The cytotoxic and growth-inhibiting effects of adrenal cortical steroids on various cell strains growing *in vitro* have been observed frequently(1-5). Recently we reported that hydrocortisone inhibited cell growth in proportion to the concentration used(6). The purpose of the present study was to determine the quantitative effects of hydrocortisone on protein synthesis and on nuclear multiplication and to investigate the qualitative effects on enzymes of cells in tissue culture.

Methods. A strain of cells derived from human liver by Chang(7) was grown in stock culture in 95% medium 199 and 5% horse serum. The cells were removed from the flasks by replacing the growth medium with 0.0125% trypsin in Hanks' balanced salt solution(8) for 15 minutes at 35°C and centrifuging the resulting cell suspension for 5 minutes at 1000 rpm. The supernatant was removed, the cells were washed in medium 199, and resuspended in medium 199 with 5% horse serum in a concentration of 100,000 cells/ml. Five ml aliquots were placed in T-flasks for studies on the rates of nuclear multiplication and protein synthesis. All cultures were incubated at 37°C for 24 hours, after which the culture medium was removed from the flasks and the flasks rinsed with 5.0 ml balanced salt solution. Determinations of protein nitrogen content of each of 3 cultures were made with a phenol reagent according to the method of Oyama and Eagle(9). Crystalline bovine albumin was used as a standard. The number of nuclei was determined in 4 different samples from each of 3 flasks by the method of Sanford *et al.*(10) using 6% citric acid to remove the cytoplasm and 0.1% crystal violet to stain the nuclei.

The culture medium to be tested was added to the remaining flasks; 6 flasks were used for each group.

At the end of the experiment 3 flasks from each group were used for determination of protein content and 3 flasks for the number of nuclei.

For microscopic studies coverslips were placed into Leighton tubes containing 100,000 cells suspended in 1 ml of medium 199 with 5% horse serum. After incubation for 24 hours at 37°C, the culture medium was

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