We are indebted to Dr. David F. Bohr for valuable advice and assistance in this study and to Mr. Richard Jalinek and Mr. Ralph King for carrying out the calculations on a digital computer.

1. Muirhead, E., Stirman, J., Lesch, W., Jones, F., *Surg. Gynec. and Obst.*, 1956, v103, 673.

2. Merrill, J. P., *J. Am. Clin. and Chem. Assoc.*, 1958, v70, 81.

3. Gomez, A. H., Hoobler, S. W., Blaquier, P., *Circ. Res.*, 1960, v8, 464.

4. Friedman, M., Freed, S. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 670.

5. Hajdu, S., Leonard, E., *Circulation*, 1961, v24, 530.

6. Muirhead, E., Jones, F., Stirman, J. A., *J. Lab and Clin. Med.*, 1960, v56, 167.

7. Muirhead, E., Hinman, J. W., Daniels, E., Kosinski, M., *Fed. Proc.*, 1963, v22, 181.

8. Ledingham, J. M., Cohen, R. D., *Clin. Sci.*, 1962, v22, 69.

9. Blaquier, P., Taquini, A. C., personal communication.

Received September 3, 1963. P.S.E.B.M., 1964, v115.

Induction of Tolerance to Skin Grafts in Mice with Disrupted Liver and Kidney Cells.* (28814)

W. D. KELLY, J. M. SMITH, C. MARTINEZ AND R. A. GOOD
Departments of Surgery, Physiology and Pediatrics, University of Minnesota, Minneapolis

A specific state of tolerance to homografts across both weak and strong histocompatibility barriers may be induced in adult inbred mice by a variety of techniques including parabiosis and injection of large quantities of cells of donor origin(1-13). More recently it has been shown that cell-free antigenic material prepared by cell disruption is similarly capable of inducing tolerance in adult mice (14-18). This finding may be considered as further supporting the views previously expressed from this laboratory that fundamentally all experimental techniques for induction of tolerance are similar and that the main factors are antigenic disparity between host and donor, the relationship between quantities of donor antigen and mass of immunologically competent cells in the host, and rate and duration with which antigen is presented to the host. Agents such as X-radiation or drugs that suppress immune response may be viewed as means whereby the mass of lymphoreticular tissue is reduced or is prevented from proliferating in response to antigen. Under these circumstances relatively small quantities of antigen may be sufficient to overwhelm or exhaust the immunologically competent cells of the host with the resultant induction of tolerance. Contrariwise, the same end may be achieved if enormous doses of antigen can be injected into the host so that the lymphoreticular cell mass is overwhelmed in spite of its ability to proliferate in response to antigen. The latter technique would have much to recommend it clinically as well as experimentally since it would avoid the generalized and non-specific destructive effects of irradiation or suppressive drugs on the host including such undesirable complication as increased susceptibility to infection, impairment of general nutrition and damage to reproductive and other tissues. Even more important, such an approach using disrupted cellular material would obviate the occurrence of a graft-*vs*-host reaction attributable to viable immunologically competent cells with its attendant morbidity and mortality. The technique of employing disrupted cell materials as the tolerance-inducing antigenic source is admittedly more difficult than the use of intact replicating cells since larger amounts of material must be used and this material must be presented to the recipient over longer periods. In addition, the necessary quantity of antigen may be essentially unattainable if one has to rely on the use of spleen or bone marrow cells in non-inbred lines of animals since here all the antigen must be obtained from one donor. It seems likely that the antigens involved in histocompatibility re-

* Aided by grants from Am. Cancer Soc. and U.S.P.H.S.

action are present in most if not all nucleated cells. There is also some evidence that at least some of these antigens may be present on erythrocytes(19). It therefore becomes of importance to study the efficacy of other tissues in the body to contribute the antigenic materials capable of inducing tolerance following cellular disruption. This report concerns investigations in which it is shown that disrupted liver and kidney tissue are as effective as disrupted spleen cells in inducing tolerance in the male-female system of histoincompatibility in C57Bl/1 mice.

Method. The system studied is that of male-female histoincompatibility originally described by Eichwald and Silmser(20) in which a male skin graft is normally rejected by the female of the same strain. This phenomenon is a consequence of the antigenic difference dependent upon the y chromosome of the male and it has been demonstrated that it conforms in essentially all details to the classical features of homograft rejection conditioned by an immune response on the part of the host(2,16,17). Adult female mice of the C57Bl/1 strain were used as recipient animals in all experiments with donor tissue being furnished by adult males of the same strain. Four groups of animals were studied as follows: Group I received injections of disrupted male spleen cells, Group II received injections of disrupted male liver cells, Group III received injections of disrupted male kidney cells and Group IV received injections of an equal volume of Ringer's solution. Donor tissues were removed from male mice freshly killed with ether. For purposes of simplicity 100 mg wet weight of fresh liver or kidney were considered equivalent to one spleen. Spleen weights varied from approximately 80 to 120 mg. Spleens and equivalent weights of liver and kidney were processed individually by mincing with scissors followed by homogenization in a glass Potter-Elvehjem tissue homogenizer. The tissue homogenates were each subjected to 5 cycles of freezing and thawing using a dry ice-alcohol bath for freezing and a water bath at 37°C for thawing. Microscopic examination of the material prepared in this way revealed no evidence of intact viable cells as judged by trypan blue staining. The disrupted cell brei was adjusted with Ringer's-lactate solution to a volume in which 0.5 ml contained material obtained from 100 mg wet weight of the starting material, except as noted below. Prior to injection the material was filtered through 2 layers of surgical gauze sponge to remove gross debris.

The first injection of material was somewhat variable since the injection of liver or kidney brei intravenously was poorly tolerated. For this reason only one-fourth of the prepared material corresponding to 25 mg wet weight in a volume of 0.25 ml was given intravenously in the liver and kidney groups with the remainder of the material prepared (75 mg in a volume of 0.75 ml) being given intraperitoneally. In the spleen group the standard routine previously established was followed administering intravenously material prepared from 1 spleen (average 100 mg wet weight) in a volume of 0.5 ml. In all groups, heparin 100 international units was given intraperitoneally 10-15 minutes prior to the intravenous injection. Controls were treated in the same way. After the first injection, all subsequent injections were uniform, each group receiving the appropriate injection intraperitoneally in 0.5 ml volume.

Two injections were given weekly spaced 3 or 4 days apart. Injections were continued for a minimum of 5 weeks when they were discontinued unless the state of the skin graft was in doubt, in which case injections were given for one additional week. Eight days after injections were started (with 3 injections having been given) full-thickness skin homografts of abdominal skin approximately 2 × 2 cm were taken from male donors and placed on the backs of the female recipients in a bed of similar size. In each instance the graft was rotated 180° from the direction of normal hair growth to aid in identification of graft survival. Grafts were fixed in place with 5-0 silk sutures and were left uncovered. Grafted animals were housed individually in plastic cages and fed Purina laboratory chow and tap water. Grafts were inspected every other day for a month and several times weekly thereafter for a period in excess of 3 months. Graft survival was scored primarily on the basis of survival of grafts in which hair growing in the reverse direction from normal was evident.

Results. The results are summarized in Table I. Disrupted liver and disrupted kidney

TABLE I. Induction of Tolerance in Adult Female C57Bl/1 Mice to Male Skin Grafts Using Disrupted Cellular Material.

	Group	No. of females accepting male skin*/No. grafted
I	Disrupted spleen†	3/6
II	Disrupted liver	9/12
III	Disrupted kidney	8/11
IV	Ringer's solution	1/9

* Evaluated 3 mo after grafting.
† An additional 9 experiments are in progress. As of 2 mo after grafting there are 5/9 grafts surviving.

cells are at least as effective as disrupted spleen cells in invoking tolerance to male skin grafts placed on C57Bl/1 adult females. The incidence of tolerance induced with disrupted spleen cells observed in these experiments is somewhat less than that previously reported from this laboratory(18). This observation may be a reflection of the use of donor mice in this experiment obtained from a separate colony of the same strain located in an adjoining building. Both colonies were originally derived from the same source but had been separated for several years.

Discussion. The experiments reported here confirm prior observations that disrupted spleen cells injected in relatively large amounts over a prolonged period are capable of inducing tolerance to male skin in female mice of the C57Bl/1 strain in a significant number of instances. The experiments also report a new finding that disrupted liver or kidney cells in amounts equivalent to the spleen are likewise capable of inducing tolerance to male skin in the female.

This is similar to observations previously reported by Linder(17) although our work differs from his in that he apparently used pooled spleen, liver and kidney as the source of tissue antigen whereas we tested individual tissues.

The ability of liver and kidney as well as spleen to induce tolerance under the present experimental conditions can be explained by the view that antigenic material involved in sex-linked histoincompatibility is present in nucleated tissue distributed throughout the body and that it is present in the liver and kidney in amounts comparable to that found in the spleen. It is of interest that the microsomal H-2 isoantigen did not seem to be demonstrable in liver tissue when homograft sensitization was used as the indicator in the studies of Manson *et al*(21). The exact mechanism by which tolerance is induced with this technique is obscure but is considered most likely to be explained by the phenomenon of antigen overloading or immunologic paralysis.

The ability to use liver and kidney as well as spleen tissue to prepare antigen for induction of homograft tolerance by an antigen overloading technique may prove to be of value in those species (including man) in which all antigenic resources must be obtained from a single individual. This approach may be particularly useful in permitting utilization of fresh cadavers as a source of both an organ for transplantation as well as large quantities of antigenic material with which to facilitate induction of a tolerant state without the hazard of a graft-*vs*-host reaction. Further studies of other tissues as antigen sources are in progress.

Summary. Acquired immunological tolerance has been established in adult C57Bl/1 female mice to male skin grafts by injection of disrupted cellular material prepared from male liver and kidney as well as from spleen.

1. Martinez, C., Shapiro, F., Good, R. A., Publishing House of Czech. Acad. Sci., Prague, 1962, p329.
2. Mariani, T., Martinez, C., Smith, J. M., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 596.
3. ———, *Ann. N. Y. Acad. Sci.,* 1960, v87, 93.
4. Shapiro, F., Martinez, C., Smith, J. M., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 472.
5. Martinez, C., Shapiro, F., Good, R. A., *ibid.,* 1960, v104, 256.
6. Nakic, B., Silobrcic, V., *Nature,* 1958, v182, 264.
7. Skowron-Cendrzak, A., Konieyna-Marczynska, B., *ibid.,* 1959, v184, 1590.
8. Rubin, B. A., *ibid.,* 1959, v184, 205.
9. Jensen, E., Simonsen, M., *Ann. N. Y. Acad. Sci.,* 1962, v99, 657.
10. Brent, L., Gowland, G., *Nature,* 1962, v196, 1298.
11. Linder, O., *Ann. N. Y. Acad. Sci.,* 1962, v99,

680.

12. Wigzell, H., *Proceedings of Liblice Symposium on Mechanisms of Immunological Tolerance,* Publishing House of Czech. Acad. Sci., Prague, 1962, p267.

13. Hasek, M., Puza, A., *ibid.,* 1962, p257.

14. Martinez, C., Smith, J. M., Blaese, M., Good, R. A., *Fed. Proc.* 1963, v22, 441.

15. Medawar, P. B., *Transplantation,* 1963, v1, 21.

16. Billingham, R. E., Silvers, W. K., *J. Immunol.,* 1960, v85, 14.

17. Linder, O., *Transpl. Bull.,* 1961, v28, 36.

18. Martinez, C., Blaese, M., Smith, J. M., Good, R. A., *Nature,* in press.

19. Kelman, H., Martinez, C., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 875.

20. Eichwald, E. J., Silmser, C. R., *Transpl. Bull.,* 1955, v2, 148.

21. Manson, L. A., Foschi, G. V., Dougherty, I., Young, T. B., *Fed. Proc.,* 1963, v22, 172.

Received September 3, 1963. P.S.E.B.M., 1964, v115.

Transaminase Changes in Rats After Exercise.* (28815)

JERRY B. CRITZ AND ARTHUR W. MERRICK (Introduced by W. O. Read)
(With the technical assistance of Peter L. Boman)
Departments of Physiology and Pharmacology, University of Missouri Medical Center, Columbia and University of South Dakota School of Medicine, Vermillion

Evidence has been accumulating since 1956 suggesting a relationship between the glutamic-oxalacetic transaminase (GOT) level and muscular or metabolic activity.

Vagotomy of the pigeon, presumably allowing an imbalance of activity in favor of the sympathetic nervous system, was found to result in increased GOT in skeletal, myocardial and hepatic tissue(1). Barbieri(2) measured the GOT content in the 4 chambers of the heart *(Bos taurus)* and reported the transaminase content of the left ventricle was highest with decreasing amounts present in the right ventricle, left auricle and right auricle. Administration of 2,4-dinitrophenol, a metabolic stimulant, resulted in an elevated GOT concentration in skeletal muscle and hepatic tissue(3). Serum glutamic-oxalacetic transaminase (SGOT) was determined in men before and after 3 three-minute rounds of boxing(4). A significant rise in SGOT was observed immediately after this type of exercise. Altland and Highman(5) forced rats to walk for 16 hours and found that this work load resulted in a significant increase in SGOT. Recent research indicated that untrained men, exposed to the Harvard Step Test, had a significant decrease in SGOT(6). Well-trained athletes, on the other hand, did not deplete the serum enzyme level following the same work load. The athletes, however, did have lower SGOT titers after time trials in their particular track events.

On the basis of the above evidence, experiments were designed to minimize tissue damage and to test further the exercise theories.

Materials and methods. Adult female rats of the Sprague-Dawley strain, weighing between 160 and 234 g, were fed standard Purina Laboratory Chow and given water *ad libitum.*

The exercise consisted of 2 series of swimming animals and 2 series subjected to a treadmill run. The rats swam in a glass tank (approximately 23°C) for either 15 or 60 minutes (or to exhaustion; the animal was considered exhausted if it remained below the surface for 60 seconds). The rats ran on the treadmill at a speed of 34 meters per minute. The first series ran 7 minutes, the second group ran 15 minutes. Immediately after the exercise the rat was killed by decapitation. A control animal was killed with each experimental animal. The first few drops of blood were collected in a waxed dish for determination of the hematocrit (International Hemacrit Centrifuge). The remainder of the blood was captured in a centrifuge tube. The clot was allowed to retract, then centrifuged at

* This investigation was supported in part by research grants from Nat. Inst. Health.