mulation of cholesterol and promote a rapid elimination of accumulated tissue β-sterol. It would seem that the presence of a 6α-hydroxyl group in the cholanic acid molecule has no special significance with respect to the hypocholesteremic properties of the acid.

It was of interest that no cholic acid could be detected in the small intestinal content of hyocholic and hyodeoxycholic acid-fed mice. Two possible mechanisms may explain this observation: (a) hyodeoxycholic acid may interfere with some early step in the conversion of cholesterol to cholic acid, or (b) hyodeoxycholic acid may bring about such a rapid excretion of hepatic sterols that oxidation is bypassed. A rapid excretion of hepatic sterol, resulting from administration of hyodeoxycholic acid, has been shown previously(4).

Summary. Hyocholic acid effectively prevented accumulation of dietary cholesterol in mice fed diets supplemented with cholesterol but had little or no effect on tissue cholesterol concentrations in mice fed normal diets. Treating mice with antibiotics largely reversed the effect of hyocholic acid, and permitted the accumulation of dietary cholesterol in hyocholic acid-treated mice. It was demonstrated that the hypocholesteremic effect is probably mediated by hyodeoxycholic acid, which arises from the action of intestinal bacteria on hyocholic acid. In animals which received hyocholic or hyodeoxycholic acid, cholic acid disappeared from the bile acid spectrum of the small intestine. Treatment with antibiotics restored cholic acid to the bile acid spectrum of hyocholic acid-treated mice but not to that of hyodeoxycholic acid-treated mice.

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Subcellular Transplantation Antigen Derived from Dog Spleen Cells Cultivated *in vitro*.* (29274)

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Previous work from this laboratory demonstrated that transplantation antigenic activity could be detected in the medium in which rabbit spleen cells had been cultivated in tissue culture(1,2). The antigenic activity was not removed by Millipore filtration but could be recovered by ultracentrifugation. When injected intradermally into a recipient rabbit the antigenic material was consistently able to induce transplantation immunity as indicated by the accelerated rejection of a test skin homograft from the donor of the cultured spleen. That the antigenic activity was specific for the spleen donor could be shown by histologic comparison of test skin homografts from the spleen donor and from an indifferent donor applied to the same antigen recipient, and by the fact that the antigenic preparation had no effect when injected back into the spleen donor. Following Millipore filtration the antigenic material was obtained as a fine suspension which was non-toxic upon intravenous infusion. This rabbit transplantation antigen (or antigens) appeared to be significantly more stable than several trans-

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plantation antigens recently extracted from mouse tissues by other workers(3,4,5).

With this work as a background it was decided to determine whether or not a similar approach might yield significant amounts of active transplantation antigenic material from other species. The dog was selected for the present study because its large size and relative availability have made it the animal most commonly used in experimental organ transplantation. The ultimate goal of these investigations was recovery of non-toxic subcellular antigenic material which might be of use in the attempted production of adult immunological tolerance.

Materials and methods. Adult mongrel dogs weighing between 10 and 20 kg were used as experimental animals. The animals were caged individually in an air conditioned animal room and were maintained on a diet of commercial dog food and water. All surgical procedures were carried out under sodium pentobarbital anesthesia (30 mg/kg) and sterile technique was used throughout. Spleens for tissue culture were excised through a standard midline laparotomy incision. Immediately prior to excision the majority of blood was evacuated from the spleen by injection of 1.0 ml of 1:1000 epinephrine into the splenic artery. Following excision the spleens were teased apart in Eagle Hela medium containing 20% normal dog serum. The resultant suspension was passed through a coarse wire mesh screen and was placed in tissue culture in plastic bottles, as described previously for rabbit spleen cells(1,2), using Eagle Hela‡ medium with 20% dog serum, penicillin and streptomycin. Incubation temperature was maintained at 37°C. Periodic cultures to check for bacterial contamination were taken and any tissue culture bottles suspected of being contaminated were discarded. Approximately 50 tissue culture bottles were used for each dog spleen.

After 48 hours growth *in vitro* (the minimum time previously shown to produce a high yield or rabbit antigenic material) the culture medium was collected from all of the bottles containing explants from a single donor dog spleen. The pooled culture medium was centrifuged at 1500 $\times$ *g* for 15 minutes to remove all cells and gross cellular debris. Following centrifugation the medium was passed through a Millipore§ filter of 0.45 micron pore size and was then spun in a Spinco model L preparative ultracentrifuge at 30,000 $\times$ *g* for 1 hour. The resultant amorphous precipitate termed the "ultracentrifugal antigenic concentrate" was suspended in a small volume of Eagle Hela medium and was injected intradermally into a recipient animal to test for antigenic activity.

Kidney homotransplantation was carried out by suturing the renal artery and vein of the donor kidney end-to-end to the common carotid artery and external jugular vein of the recipient. The ureter was cannulated with a polyvinyl tubing and was then brought out through a stab wound as a skin ureterostomy. Urine was collected in plastic Fenwal‖ bags held in place by canvas jackets. Total ischemia time for the transplanted kidneys was less than 40 minutes in all experiments.

Results. Control animals. In 5 experiments the ultracentrifugal antigenic concentrate obtained from a single cultured spleen was injected intradermally back into the spleen donor. Forty-eight to 72 hours later the donor animal received a kidney homotransplant from an unrelated dog. All transplants had a good urinary output initially and there were no technical failures in this group. Rejection of the kidney homotransplants was considered to have occurred when function ceased as indicated by the onset of anuria unresponsive to rapid intravenous infusion of 500-800 ml of 5% dextrose and water. The functional survival of kidney homotransplants in this control group of animals is listed in Table I. Mean survival was 5.8 $\pm$ 1.1 days. This is not significantly different from the functional survival of homotransplanted kidneys in untreated animals previously described by Egdahl and Hume(6) (average survival 5.9 days).

Experimental group. In this series of 7 experiments the ultracentrifugal antigenic concentrate obtained from the culture of a single spleen was injected intradermally into

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§ Millipore Filter Corp., Watertown, Mass.

‖ Macbick Co., Cambridge, Mass.

TABLE I. Survival of Kidney Homotransplants. Control Group

Dog No.	Survival of kidney transplant (days)	Histologic appearance of kidney transplant
101	7	Massive round cell infiltrate, moderate tubular destruction, cortical hemorrhage, edema.
102	5	Moderate round cell infiltrate, incipient tubular destruction, no hemorrhage, edema.
103	5	Mild round cell infiltrate, marked tubular destruction, no hemorrhage, edema.
104	5	Marked round cell infiltrate, moderate tubular destruction, no hemorrhage, edema.
105	7	Marked round cell infiltrate, marked tubular destruction, no hemorrhage, edema.

Mean = 5.8 ± 1.1 (S.D.).

an unrelated, homologous animal. Forty-eight to 72 hours later the antigen recipient received a kidney homotransplant from the original spleen donor. There was one technical failure in this group because of thrombosis at the arterial anastomosis. The functional survival of the 6 remaining kidney homotransplants was determined as before. The results are listed in Table II. Mean survival in this group was 3.0 ± 0.6 days, significantly shorter than the survival of kidney homotransplants in the control group of animals, $p<0.01$. As shown in Fig. 1 and Table II the histologic appearance of kidney transplants, excised from several animals in the experimental group at the time of anuria, was consistent with a second set or hyperimmune rejection response.

TABLE II. Survival of Kidney Homotransplants. Experimental Group

Dog No.	Survival of kidney transplant (days)	Histologic appearance of kidney transplant
371	3	Moderate round cell & polymorphonuclear infiltrate, moderate hemorrhage, moderate tubular destruction, edema.
313	4	Mild round cell infiltrate, mild hemorrhage, moderate cast formation and tubular destruction, edema.
249	2	Mild round cell infiltrate, moderate hemorrhage, massive tubular destruction, edema.
403	3	Very few cells, moderate hemorrhage, massive tubular destruction, edema.
372	3	Massive round cell infiltrate, moderate hemorrhage, moderate tubular destruction, edema.
392	3	Moderate round cell & polymorphonuclear infiltrate, no hemorrhage, moderate cast formation and tubular destruction, edema.

Mean = 3.0 ± 0.6 (S.D.).

FIG. 1. Histologic section of kidney transplant removed at time of anurea 3 days after transplantation from a dog in experimental group. Destruction of tubular cells and peritubular hemorrhage are evident. Appearance is consistent with a second-set rejection response. (H and E, × 400.)

In a final series of 4 experiments, the pooled medium from the culture of a single donor spleen was passed through a Millipore filter as before and was then infused immediately intravenously into a homologous recipient animal without concentration of the antigenic material by ultracentrifugation. When infused intravenously the Millipore filtered antigenic medium was apparently entirely non-toxic.

Discussion. The experiments reported here indicate that an active subcellular transplantation antigen or group of antigens can be harvested from the tissue culture medium in which dog spleen cells have been cultivated *in vitro*. That the accelerated rejection of donor kidney homotransplants seen in anti-

gen treated recipients was not a non-specific effect is indicated by the fact that injection of the anitgenic material back into the spleen donors did not induce accelerated rejection of kidney homotransplants from indifferent animals.

To our knowledge the isolation of a subcellular, transplantation antigenic preparation from the dog has not previously been reported. The fact that cultures of both rabbit (1,2) and dog spleen have yielded media with similar antigenic activities suggests that the release of transplantation antigenic material into the surrounding fluid may be a general result of the cultivation of mammalian spleen cells *in vitro*.

It now appears likely that the intravenous route is the most effective for induction of tolerance(3,7). Since the Millipore filtered dog antigenic medium is apparently entirely non-toxic upon intravenous infusion it may be potentially useful in future experimental attempts to induce adult immunological tolerance to organ and tissue grafts when used in combination with short courses of immunosuppressive drug therapy, or sublethal total body radiation.

Summary. 1. Millipore filtered tissue culture medium in which dog spleen cells have been cultivated *in vitro* was found to contain subcellular material with transplantation antigenic activity which could be concentrated by ultracentrifugation. Antigenic activity was demonstrated by the accelerated rejection by antigen recipients of a kidney homotransplant from the original spleen donor. 2. The antigenic material did not induce accelerated rejection of kidney homotransplants when injected back into the original spleen donor. 3. The Millipore filtered culture medium containing antigenic activity was apparently nontoxic when infused intravenously into homologous recipients.

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Influence of Mercaptoethanol Treatment on Skin Sensitizing and Complement Binding Ability of 7 S Anti-Dinitrophenol-Bovine Gamma Globulin Antibody.* (29275)

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Rabbit 7 S immune globulin can be split by papain digestion(19) into two fragments containing the specific antigen combining activity, and a third fragment, which has no antigen combining sites, but which is essential for complement fixation(22) and skin sensitization(17). This third fragment is the one involved in precipitation of aggregated 7 S gamma globulin by rheumatoid factor(4). It was previously shown that mercaptoethanol (ME) treatment of 7 S immune globulin affects its complement binding capacity without diminishing its reactivity with rheumatoid factor(23).

In the present study the influence of ME on the skin sensitizing and the complement fixing ability of 7 S antibody and the role of complement in passive cutaneous anaphylactic

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