strated by this series of experiments.

DMSO has been shown to protect bull spermatozoa during freezing and thawing(3). Our results suggest that it may be a satisfactory medium for preservation of other tissues for prolonged storage at low temperatures. Meryman(4) has recently indicated that DMSO may be superior to glycerin in the protection of living cells during freezing, because of its more rapid passage through cell membranes.

Summary. Thyroid tissue from 8 adult dogs was autotransplanted successfully into the cerebral cortex. Histologic viability was demonstrated in all instances for as long as 2 months. Thyroid tissue from 6 adult dogs was frozen at –196°C after immersion in dimethyl sulfoxide and stored at this temperature for 2 weeks. After rapid thawing, the tissue was autotransplanted into the cerebral cortex. Histologic examination showed apparent viability of the transplanted tissue at 6 weeks (one animal) and 12 weeks (2 animals) after autotransplantation.

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Serial Transfer of Antibody Producing Cells on the Chorioallantoic Membrane.* (28281)

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The growth of distinct foci of cells on the chorioallantoic membrane (CAM) of embryonated eggs following inoculation of adult buffy coat or spleen cells (Simonsen phenomenon) has been well described(1). Recent studies with inbred lines of chickens have established that this phenomenon is a *graft vs host* reaction(2). The replication on the CAM of the adult cells responsible for formation of these foci is evident from the success of serial transfers of the foci and from the direct identification by chromosomal analysis of proliferating donor cells(3). The formation of humoral antibodies by adult chicken cells on the CAM has also been reported(4). Since the *graft vs host* reaction is presumed to be an immunologic event with the adult cells responding to the presence of a host tissue antigen, it appeared reasonable to attempt the serial transfer on the CAM of chicken cells capable of antibody formation to a defined antigen. Our results indicate that, although formation of foci continued through 3 transfers and antibody production continued for at least 2 transfers, an increase in antibody formation by exposure of the cells to specific antigen was not accomplished.

Methods. Non-inbred adult chicken hens and fertile eggs were supplied through a local hatchery. Adults were fed chicken mash and water *ad libitum.* Eggs were maintained at 37°C in a humid atmosphere before and after inoculation.

Immunization of adult chickens was by intraperitoneal injection of bovine gamma globulin (BGG). Chickens were bled from the wing vein; the spleens were removed immediately following sacrifice and tissue minces were prepared in balanced salt solution plus glucose by forcing the spleen through a number 16 stainless steel screen. Penicillin (500 μg/ml) and streptomycin (125 μg/ml) were used routinely in the medium.

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Recipient embryo eggs were ordinarily 10 to 12 days old although occasionally eggs as young as 9 days or as old as 14 days were used. The CAMs were dropped, cells introduced (with or without antigen) and shell punctures sealed with melted paraffin. Eggs were incubated for 5 days after inoculation and the CAMs were removed and stored frozen at −20°C. The CAMs were thawed, homogenized in a tissue grinder and clarified by centrifugation, usually within a week and always within a month after freezing.

For serial transfer of cells, the portion of the CAM containing the foci was removed aseptically, minced, and mixed at 37°C with a 0.1% trypsin solution for approximately one hour. The cells were centrifuged lightly and resuspended in fresh medium for inoculation into eggs.

Antibody was detected by the hemagglutination of chicken erythrocytes which had been coupled with BGG by means of formaldehyde(5), and titers expressed as the reciprocal of the maximum dilution showing agglutination. Control inhibition titers were run on all positive fluids and only those titers above 1:16 which were also inhibited by excess BGG are reported.

Results. Characteristic cellular foci were seen on the CAM within 5 days following inoculation of either spleen or buffy coat cells. Foci were formed following 3 serial transfers, the first being the initial inoculation of the CAM with adult buffy coat cells. The number of foci appeared to decrease with each passage but no attempt was made to quantitate this number. After 5 days, the eggs were harvested and the CAMs analyzed for antibody (Table I). When the CAM was inoculated with buffy coat obtained 4 days after primary antigenic stimulation, no antibody was found in 24 eggs studied. Following recall immunization (Table I) only 4 of 68 eggs inoculated with buffy coat cells revealed antibody. In both experiments all the membranes showed cellular foci.

In contrast to this, when the CAM was inoculated with spleen cells taken from one hen 3 days after a recall injection of antigen, 67% had significant titers. For this reason the remainder of the experiments was done with adult spleen cells.

TABLE I. Antibody Production on CAM by Adult Chicken Cells.

Days after primary antigen	No. of eggs	No. of eggs with antibody*	Mean log titer†
Adult chicken buffy coat cells			
4	24	0	
Days after recall antigen			
2	17	0	
4	14	0	
7	19	2	.158
10	12	2	.275
15	7	0	
Adult chicken spleen cells			
3	12	6	.902
Days after recall antigen			
3	12	8	1.179

* Titers above 1:16.
† Negative fluids given arbitrary value of 0.

In a subsequent experiment the number of eggs with significant titers fell from 80% on the initial inoculation (transfer I) to 31% after the second transfer (Table II). It was anticipated that the continued presence of the antigen might be required for the persistence of antibody formation. However, even on the initial inoculation the presence of antigen in the egg appeared to mask the detection of antibody. These observations are summarized in Table III.

To attempt the serial transfer of antibody producing cells in the presence of antigen and to detect their presence on the CAM, spleen cells obtained after a recall injection of BGG were inoculated into eggs together with 500 μg BGG N per egg. Following each transfer, a portion of recipient eggs was inoculated

TABLE II. Antibody Production by Spleen Cells In Absence of Antigen.

Transfer	No. of eggs	No. of eggs with antibody	Mean log titer
I	10	8	1.415
(Chicken→Egg) II	13	4	.463

TABLE III. Antibody Production on CAM by Immune Adult Chicken Spleen Cells in Presence of Antigen.

Bovine gamma globulin added to CAM	No. of eggs	No. of eggs with antibody	Mean log titer
100 μg N	7	0	0
10 "	6	2	.702
1 "	3	2	1.204
0	7	5	1.631

without added antigen to allow the expression and detection of antibody. The remainder received 500 μg BGG N and served to continue the cell line. As is summarized in Table IV, the percentage of eggs with significant antibody titers fell rapidly and by the third transfer only one egg with a very low titer showed activity. All of the membranes analyzed showed the presence of foci. Analysis of representative eggs inoculated with cells and antigen showed virtually no activity as we would expect from the earlier experiment (Table III). Analysis of the CAM alone, the CAM inoculated with spleen cells from a non-immunized chick or the CAM 5 days after injection with antigen alone revealed no antibody. The introduction of heterologous spleen cells (guinea pig) produced no foci in recipient eggs.

Discussion. Considerable evidence has accumulated in recent years that cell proliferation is a requisite of antibody formation (6-8). Since the formation of Simonsen foci on the CAM requires proliferation of cells with immunologic competence, these experiments were performed in the hope that the CAM might provide the environment necessary for induction of antibody formation by antigen.

TABLE IV. Loss of Antibody-Producing Activity By Spleen Cells During Serial Transfer in Presence of Antigen.

Transfer	No. of eggs	No. of test eggs with antibody	Mean log titer
I	8	5	1.314
II*	22	5	.342
III†	10	1 (titer 1:19)	.279

* Antigen injected with first transfer but not with second.

† Antigen injected with first and second transfers but not with third.

Despite the fact that serial transfer of foci and continued but diminishing antibody formation were achieved, the amount of antibody produced was not increased by simultaneous injection of 1 to 100 μg antigen nitrogen with the transferred cells. The larger amounts of antigen reduced the amount of antibody detected, presumably by neutralizing it on the CAM. Exposure of the cells to antigen on one transfer did not increase the antibody detected on a subsequent transfer where the cells were grown without antigen.

Our interpretation of these findings is that induction of antibody formation by antigen involves 3 distinct processes: (1) cell replication (2) cell maturation to a specific antibody-forming cell and (3) continued production of antibody by the mature cell. Antigen appears to be required for only steps (1) and (2), but the environment of the CAM is appropriate for only steps (1) and (3).

Another interpretation is that there are several classes of cells with immunologic competence and that those leading to the Simonsen phenomenon are different from those leading to formation of extracellular antibody. In this case the formation of foci does not provide evidence of the replication of cells with antibody-forming competence. The detection of such replication may require transfer of the cells back to an adult environment.

Summary. Serial transfer of foci on the CAM through at least 3 transfers is confirmed. Significant titers of antibody were found in a high percentage of recipient eggs 5 days after inoculation of spleen cells from an immunized chicken. The transfer of cells from the CAM of these eggs to other eggs resulted in development of foci, but the proportion of eggs with detectable antibody declined rapidly after the first transfer. The presence of antigen in the eggs inoculated with spleen cells from immunized chickens did not stimulate antibody formation. Finally, although both spleen cells and buffy coat cells yielded large numbers of foci, formation of antibody on the CAM was consistently greater with the inoculation of spleen cells from immunized donor chickens.

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Inhibitory Effect of 3-Methylcholanthrene on Induction of Massive Necrosis of Adrenal Cortex by 7,12-Dimethylbenz(a)Anthracene.* (28282)

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Evidence has been reported(1) indicating that the mechanisms by which 7,12-dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (3MC) inhibit corticosterone synthesis in the adrenal gland are not the same. It was suggested that depletion of adrenal corticosterone in DMBA-treated rats is secondary to DMBA-induced acute necrosis of the adrenal cortex, since dead cortical cells cannot synthesize hormones. The mechanism by which 3MC suppresses corticosterone synthesis was believed to be an inhibition of the biosynthetic pathway leading to corticosterone. The exact nature of this mechanism has yet to be elucidated.

Currie *et al.*(2) recently reported that 2-methyl-1, 2-bis (3-pyridyl)-1-propanone (Metopirone, or Ciba SU 4885) given prior to DMBA inhibited DMBA-induced acute necrosis and hemorrhage of the adrenal cortex in rats. Metopirone is known to interefere with the synthesis of cortisol and corticosterone by inhibition of 11β-hydroxylation(3). This interesting observation led us to believe that since 3MC inhibits corticosterone synthesis, it should exert an inhibitory effect on production of adrenal necrosis by DMBA. In the present investigation, it was shown that 3MC given to rats prior to DMBA could indeed prevent the induction of acute necrosis and hemorrhage by DMBA. Furthermore, the mechanism by which 3MC inhibited corticosterone synthesis in the adrenal cortex appeared to be similar to the one by which Metopirone inhibited it.

Materials and methods. The experimental animals were female Sprague-Dawley rats 55 to 60 days old and weighing 165 to 185 g. The carcinogens were dissolved in olive oil in a concentration of 30 mg/ml for 3MC, and 20 or 30 mg/ml for DMBA. They were fed to the rats through a stomach tube in all experiments. Metopirone was dissolved in olive oil in a concentration of 10 mg/0.1 ml, and was injected into the rats intraperitoneally.

There were 10 groups of experimental animals, each containing 10 rats: (a) controls, (b) rats receiving 20 mg/ml of DMBA, (c) rats receiving 30 mg/ml of DMBA, (d) rats receiving 10 mg of Metopirone intraperitoneally every 4 hours for 3 doses, (e) rats given 30 mg/ml of 3MC daily for 2 consecutive days and then a single dose of 20 mg/ml of DMBA 48 hours later, (f) rats given 30 mg/ml of 3MC daily for 2 consecutive days and then a single dose of 30 mg/ml of DMBA 48 hours later, (g) rats given 10 mg of Metopirone every 4 hours for 3 doses and then a single feeding of 20 mg/ml of DMBA 4 hours

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