jury of the esophageal mucosa.

Discussion. This two-stage esophagostomy is a safe operative procedure, and the animals have been found to satisfy their nutritional requirements by the natural processes of eating and drinking. 70-90% of food swallowed passes directly into the stomach, and the portion which escapes through the esophagostomy, if accessible to the animal, may be eventually consumed.

Four dogs equipped with this type of esophagostomy have been maintained in good general condition for more than a year, and the time spent on their care was hardly greater than that required for dogs without esophagostomy. The 3 cm esophagostomy was small enough to make the animal self-sufficient with regard to feeding, and large enough to permit satisfactory eversion of a flap of esophageal mucosa in experiments requiring the exclusion of saliva from gastric secretions. It is of interest that duodenal reflux, as evidenced by the appearance of bile in the gastric collection, was very infrequent in all our preparations with a gastric fistula placed on the most dependent part of the anterior surface of the body.

In our experience, gastric fistula supplemented with the described esophagostomy provides the simplest and most satisfactory preparation for the collection of gastric secretion free of salivary contamination, and is eminently suitable for the study of secretagogue or inhibitory activities of various substances administered parenterally. This type of esophagostomy may also be utilized for mild or moderate stimulation of the cephalic phase of gastric secretion by "limited" sham feeding, a procedure whereby the animal is fed for a few minutes with small single strips of meat. The preparation, however, is less suitable for maximal cephalic phase responses by prolonged unrestricted sham feeding. The latter may be associated with esophageal movements sufficiently violent to retract the everted mucosal flap through the esophagostomy, and thus allow the passage of small amounts of food into the lower esophagus and stomach.

Summary. A safe two-stage operative technic is described for establishing an esophagostomy in the dog which permits natural feeding. When required for experimental purposes, a simple procedure allows this esophagostomy to assume, temporarily, the advantages of the standard double-barrelled esophagostomy.

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Production of Antibodies by Adult Hen Spleen Cells Transferred to Chick Embryos.* (23810)

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It is generally accepted that the chick embryo is incapable of producing antibodies(1). A wide variety of cells and tissues have been successfully grafted onto the chorioallantoic membrane (CAM) of the developing chick embryo. The purpose of the work reported here was to determine whether, and under what conditions, adult hen spleen cells, transferred to CAM, would produce antibodies.

Materials and methods. In all experiments Wynhamp or New Hampshire Red hens (ca 2 kg) were used as donors. Bovine serum albumin (BSA), Armour's Fraction V, in Hanks or in phosphate buffered (pH 6.4) saline solution (PBS), was the antigen used. The donor hens were killed by decapitation and the

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spleens removed aseptically. The spleen capsules were removed and the pulps pressed through surgical gauze (28x24 threads per inch). The pulp was placed in 3 ml of Hanks solution in graduated centrifuge tubes. The volumes of pulp obtained, as indicated by the final volume of fluid, ranged from 1.1 to 3.5 ml. These suspensions were used as such, or in some experiments the buffy coats were separated by centrifugation and the white cells resuspended to the original final volumes in Hanks solution. Portions of the suspensions were preserved for antibody determinations and 0.1-ml amounts were inoculated by syringe and needle directly onto the CAM's of varying numbers of 9- to 10-day-old embryonated Hyline (934A) eggs. The eggs were reincubated for various periods and the CAM's in the region containing the cells (ca 20 mm diameter) were removed. The fluids were expressed from the membranes and transplanted cells by tissue press(2) and collected in graduated capillary pipettes. The original spleen or white cell suspensions were ground in mortars with PBS. Antibodies in both types of fluids were determined by the hemagglutination technic of Boyden(3). Erythrocytes were sensitized by solutions of BSA containing 0.25 mg/ml. Specificity of the reactions was controlled by inhibition by specific antigen(4), using 0.2 mg BSA per ml of test fluid.

Results. Preliminary experiments were done to determine the possible distribution of antibody which might be formed on the CAM. High-titered anti-BSA rabbit serums were dropped onto the CAM's of 10-day-old embryos and the relative proportions of antibody recovered from the various tissues and fluids measured after incubation for periods of 1 to 6 days. The major portion of the antibody was, in almost all cases, found in the CAM's in the area of injection. The remainder of the CAM's contained about one-fourth as much as the local area, and the embryos, allantoic fluids, and amniotic fluids little or none. Similar results were obtained using an anti-BSA hen serum, but the recovery efficiency was not as great because the serum used was of low titer. Because of these findings, it was decided to limit the titration of antibodies to the fluids obtained from the inoculated cells and the CAM area involved.

While the literature given above is quite conclusive that embryonic tissues alone cannot produce antibodies, we took the precaution of determining, with the antigens and methods to be used, that no antibody would be produced by the embryonic tissues in the absence of transplanted cells. A total of 27 10-day-old embryonated eggs was inoculated on the CAM with 0.01 to 1 mg of BSA and incubated for 6 to 8 days. The CAM fluids were titrated but no trace of antibody was found in any.

Transfer of normal adult spleen cells to CAM's. Cells incubated with antigen before inoculation. Spleen suspensions from 4 normal donor hens were incubated *in vitro* with BSA (40 μg/ml of packed cells) for 30 minutes at 37°C. The cells were washed once with Hanks solution, and 0.1-ml amounts were inoculated onto the egg membranes. None of the transplants to the 31 surviving embryos showed any antibody after 4 to 7 days incubation.

Simultaneous inoculation of cells and antigen onto CAM's. 0.1-ml amounts of spleen suspensions from 6 normal hens were inoculated onto the membranes of embryos along with various amounts of antigen (4 μg-200 μg). Transplants on the 30 surviving embryos showed no antibody after 6 to 8 days incubation.

Transfer of spleen cells 1 to 2 days after one injection of antigen. No other antigenic stimulation. Thirteen donor hens were given 32 mg/kg of BSA intravenously, and the spleens removed after 2 days. Spleen suspensions were inoculated onto the CAM's of embryonated eggs, and the antibody titers of the fluids from the transplants and adjacent CAM's measured after varying periods of incubation. The results are summarized in Table 1. Approximately one-third of the transplants showed definite antibody after 4 to 7 days incubation. Although the titers were variable, many of them were high enough to leave no doubt of their significance. No antibodies were detected after 2 to 3 days incubation. The original cell suspensions inoculated in no case showed any detectable

TABLE I. Antibody Production by Whole Spleen Transplants from Hens Given Intravenous BSA 48 Hours Previously.

No. of donor hens	Incubation time of eggs after cell transfer (days)	No. of transplants producing antibodies	Total No. of embryos surviving	Mean log titers of positives	Range of positive log titers
2	2	0	13		
1	3	0	5		
9	4	20	48	1.57	.70–2.95
9	5	14	38	1.75	1.08–2.76
5	6	6	20	2.17	1.26–2.89
3	7	4	12	1.81	1.20–2.48

Donor hens were given 32 mg BSA/kg body wt.
Transplants from individual donors were incubated for several different time periods. All spleen suspensions were devoid of detectable antibody at time of transfer.

antibody. Not included in the table are the results from experiments using 2 donor hens whose spleens were removed 24 hours after intravenous antigen injection and the recipient embryos incubated for 5 to 7 days. None of the transplants of the 17 surviving embryos showed any antibody in contrast to the one-third positives in the 5- to 7-day transplants from donors injected 48 hours previously, shown in the table.

In a second group of experiments using white cell suspensions from spleens removed 36 and 48 hours after intravenous antigen injection, definite evidence of antibody production was also found, as shown in Table II.

Transfer of spleen cells 2 days after reinjection of antigen. No other antigenic stimulation. One hen was given a total of 185 mg of BSA intravenously over a period of 33 days. Two days after the final injection of 20 mg, the spleen cells were transferred to eggs which were incubated for 2 to 6 days. Extracts of the spleen suspensions contained antibody (log titer 1.45). Transplants and adjacent membranes from 5 of the 10 surviving embryos showed antibody (log titer 0.78-1.75). In 2 cases (2 and 6 days incubation) the antibody recovered was significantly greater than the amount inoculated. One hen, given 20 mg BSA intravenously 12 days after primary intramuscular inoculation of 0.5 mg of alum-precipitated BSA, was killed 2 days later. The spleen suspensions contained no antibody. Four of the transplants on the 7 surviving embryos (2 to 6 days incubation) contained measurable antibody (range of log titer 0.60-1.90).

Transfer of spleen cells more than 7 days after one or more antigen injections. Cells incubated with antigen before inoculation. Spleen suspensions from 6 donor hens which had received one intravenous injection of 32

TABLE II. Antibody Production by Spleen White Cell Transplants from Hens Given Intravenous BSA 36 or 48 Hours Previously.

Time spleen removed after antigen inj. (hr)	No. of donor hens	Incubation time of eggs after cell transfer (days)	No. of transplants producing antibodies	Total No. of embryos surviving	Mean log titers of positives	Range of positive log titers
36	2	4	0	8		
	6	5	1	22	1.60	
	4	6	1	15	3.34	
	2	7	0	9		
48	6	4	8	23	1.94	1.26–2.76
	5	5	6	18	2.30	1.57–2.76
	4	6	3	13	2.39	1.90–2.81
	1	7	1	2	2.78	

Donor hens were given 32 mg BSA/kg body wt.
Transplants from individual donors were incubated for several different time periods. All white cell suspensions were devoid of detectable antibody at time of transfer.

mg/kg of BSA, 7 to 28 days previously, were incubated *in vitro* with BSA (40 μg/ml of packed cells) for 30 minutes at 37°C. The cells were then washed with Hanks solution, and 0.1-ml amounts inoculated onto the CAM's, and incubated for 2 to 5 days. Of 62 surviving embryos, 5 showed low antibody titers which undoubtedly represented only transferred antibody, since the spleen suspensions inoculated contained antibody. Calculation of antibody units inoculated and recovered indicated that there was no increase of antibody in the eggs. Similar experiments were done with 2 donor hens which had received two intravenous injections 30 and 15 days previously. The spleen suspensions were incubated as above with BSA, inoculated, and the eggs incubated for 1 to 3 days. Seven embryos survived, none of which showed any trace of antibody.

Simultaneous inoculation of cells and antigen onto CAM's. Six hens were given one intravenous injection of BSA, 32 mg/kg, and killed 27 to 40 days later. 0.1-ml amounts of spleen suspensions were inoculated onto the CAM's, and then varying amounts of BSA (4 μg-200 μg) were dropped onto the cells. The eggs were incubated for 5 to 7 days. None of the transplants on the 45 surviving embryos showed any trace of antibody. Two hens were given several intravenous injections of BSA (totalling 152 and 146 mg) and killed 68 and 265 days after the last injection. Eggs inoculated with the spleen suspensions and antigen (4 μg to 200 μg) were incubated for 5 days. None of the transplants on the 25 surviving embryos produced any detectable antibody.

Discussion. It was clearly demonstrated that antibodies were produced by the whole spleen or spleen white cell suspensions transferred to CAM's, without further contact with antigen, from adult donor hens 36 to 48 hours after a single intravenous injection of BSA. Extracts of the suspensions never contained demonstrable antibodies at the time of transfer, while in many instances the CAM-transplant fluids contained significant concentrations of antibodies. The possibility that the embryo tissues were enabled to produce antibodies by factors supplied by the adult cells, is made unlikely by the fact that no antibodies were demonstrated in the membranes onto which normal adult cells and antigen had been inoculated. Cells transferred 48 hours after *reinjection* of the adult donor hens also produced antibodies. While antibody was usually present in the suspensions transferred, the amounts of antibody recovered from the CAM-transplants were, in a number of instances, significantly greater than the amounts transferred. These results are in agreement with the numerous reports that cells, taken from adult animals at varying periods from 1 to 10 days after a single or final stimulation by various bacterial and protein antigens, have consistently produced antibodies, when transferred without further antigenic stimulation to homologous, normal or irradiated adults, to newborn animals, and to tissue cultures(5).

No evidence of antibody production was obtained in the experiments in which cells were taken from adult hens more than 7 days after one or more antigen injections and transferred to CAM's simultaneously with antigen or after *in vitro* incubation with antigen. These results are in agreement with those of Dixon and Weigle(6), who found that cells, removed from adult rabbits 17 to 21 days after a final injection of protein antigen, did not produce antibodies when transferred to newborn animals simultaneously injected with antigen. Such cells, transferred to x-irradiated adult animals, produced antibodies if the recipients were given antigen at the same time, but not if the cells were incubated with the antigen *in vitro* before inoculation(7).

Our failure to demonstrate antibody production by spleen suspensions from uninjected adult hens, when transferred to CAM's simultaneously with antigen, or after *in vitro* contact with antigen, is comparable to the previously reported results with normal cells(6). Cells from uninjected rabbits, transferred to newborn animals simultaneously with antigen, or after contact with antigen *in vitro*, failed to produce antibodies against protein antigens. Cells from donors injected with antigen 2 hours prior to removal, essentially normal cells in contact with antigen (?), failed to produce antibodies against Shigella antigen when

transferred to newborn animals. Cells from uninjected rabbits, transferred to x-irradiated adults simultaneously with antigen, or after contact with antigen *in vitro,* produced antibodies against Shigella antigens(5), but not against protein antigens(7). Cells from uninjected animals in tissue culture are generally recognized not to produce antibodies(5), but one report(8) has indicated that cells from animals non-specifically stimulated by endotoxin produced antibodies following an *in vitro* contact with protein antigen.

Summary. Spleen cell suspensions from normal hens inoculated onto CAM's with BSA, or after *in vitro* contact with BSA, failed to produce demonstrable antibodies. Whole spleen cell and spleen white cell suspensions, taken from previously normal hens 36 to 48 hours after intravenous injection of BSA and inoculated onto CAM's, produced significant amounts of antibody without further contact with antigen 4 to 7 days after transfer. The white cell suspensions produced somewhat more antibody than did the whole spleen suspensions. Whole spleen cell suspensions, taken from hens 48 hours after intravenous reinjection of BSA, produced significant amounts of antibody, without further contact with antigen, 2 to 6 days after transfer to CAM's. Whole spleen cell suspensions, from hens stimulated by BSA injection more than 7 days previously, inoculated onto CAM's with BSA, or after *in vitro* contact with BSA, failed to produce demonstrable antibodies.

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Cholesterol Esterases. VII. Hydrolysis of Branched Chain Esters by Pancreatic Cholesterol Esterase.* (23811)

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In a study(1) on the relative specificity and activity of hydrolytic and esterifying cholesterol esterase in pancreatic extracts in a series of 13 fatty acids, it was observed that the enzyme was most active in hydrolysis with short chain fatty acid esters, and most active in esterification with long chain acids. An interesting exception to the high hydrolytic activity with short chain fatty acid esters was found with cholesterol isovalerate. This 5-carbon branched chain ester was hydrolyzed at 9% of the rate observed with the n-valeric acid ester. This markedly lower activity with a branched chain acid suggested the possibility of inhibition of the enzyme owing to steric hindrance effects. It was also shown that in a mixture of 2 esters the more slowly hydrolyzed ester competes with the more rapidly hydrolyzed one for the enzyme. In this paper we present data on the hydrolysis of a series of cholesterol esters, of fatty acids of varying chain length with differing degree, location, and size of branching by pancreatic cholesterol esterase.

Preparation and characteristics of branched chain esters. The esters were synthesized as described by Swell and Treadwell(1) and were recrystallized until melting points were

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