Discussion. The above data are in conformity with the view(1) that blood thromboplastin prepared *in vitro* is susceptible to reticuloendothelial phagocytosis. The cell-free control demonstrated that the thromboplastin was relatively stable in the suspending medium; the failure of undisturbed cells to inactivate thromboplastin indicated that an anticoagulant agent was not elaborated in the course of incubation. Measures that inhibited phagocytosis correspondingly reduced thromboplastin inactivation in the presence of the macrophages.

Failure of the cells to inactivate product I agrees with previous data(6) suggesting that this reagent is cleared *in vivo* with the participation of hepatic parenchymal cells rather than macrophages.

It is not evident why removal of radioactive thromboplastin was less effective than destruction of thromboplastic activity. Possibilities include: association of clotting activity with smaller particles, which might be more efficiently cleared than larger particles, resulting in a relatively low uptake of total lipid and radioactivity; digestion of phagocytosed material with release of radioactivity; or selective uptake from the crude cephalin of lipids known to have coagulant activity. The last explanation would fit with the relatively low content of phosphatidyl serine and ethanolamine (the coagulant lipids) in the P^{32} labelled crude cephalin(7).

All available studies are consistent with the view that laboratory blood thromboplastin is considered a foreign particle by the reticuloendothelial system. It remains to be determined whether clotting activity which forms under physiological conditions is similarly handled.

Summary. Under appropriate conditions rabbit peritoneal macrophages inactivated blood thromboplastin *in vitro*. In a similar system pulmonary macrophages were inert. The peritoneal macrophages appeared to remove radioactive thromboplastin from the medium, but the data were not as clear-cut. It was not possible to demonstrate increased oxygen uptake by peritoneal macrophages following exposure to thromboplastin since these cells appeared to have maximal oxygen consumption prior to mixing with the clotting reagent. It is concluded that reticuloendothelial cells treat laboratory blood thromboplastin as a foreign body.

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Effects of Freezing and Storage on Survival of Thyroid Autografts.* (28280)

ROY G. SHORTER, JACK L. TITUS, FREDERICK W. L. KERR AND JOHN C. CAMPBELL
Mayo Clinic and Mayo Foundation, Rochester, Minn.

Freezing of the thyroid gland under various conditions has been reported(1,2) to be followed by survival of heterotopic autotransplants for periods up to 4 weeks. Stewart and co-workers(2) indicated that addition of

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glycerol to the medium before freezing enhanced the survival of the autotransplanted tissue. To evaluate the effects of freezing and storage of thyroid tissue in liquid nitrogen without the addition of glycerol, the survival of autotransplants was studied in the dog.

Materials and methods. Fourteen adult mongrel dogs of either sex were subjected to subtotal thyroidectomy under full aseptic precautions. In 8 dogs a frontal craniectomy was performed at the same time, and a piece of autologous thyroid tissue measuring approximately 10 by 2 mm was inserted into the frontal cortex of the brain to a depth of approximately 1 cm. In the 6 other dogs similar pieces of thyroid tissue were removed and placed in 10% dimethyl sulfoxide (DMSO) in Ringer's solution in glass ampules and frozen in a liquid-nitrogen freezer (Linde Corp.). The tissue was frozen at a controlled rate of temperature decrease of 1°C per minute until −15°C was reached, then at a rate of 5°C per minute between −15 and −79°C, and then more rapidly to −196°C. After −196°C was reached, the tissue was stored in a liquid-nitrogen refrigerator for 2 weeks. At the end of this period, the 6 dogs were subjected to frontal craniectomy and then, after rapid thawing by immersion in Ringer's solution at 37°C, a fragment of autologous thyroid tissue measuring approximately 10 by 2 mm was placed into the frontal cortical tissue as in the other group of dogs.

Animals were killed approximately 4 weeks, 6 weeks, 8 weeks, or 12 weeks after craniectomy, and the tissue at the site of the graft was removed. The tissue was fixed in 10% neutral formalin and blocked in paraffin wax. Sections were cut at 7 μ and stained with hematoxylin and eosin for examination by light microscopy. A specimen from each fragment of frozen thyroid tissue to be autotransplanted was similarly fixed and sectioned after thawing.

Results. All the frozen thyroid tissues that were sectioned as controls were normal histologically.

In the 8 animals that had intracortical autografting of fresh thyroid tissue, survival of the grafted tissue was seen in all cases after all time intervals. Histologically well-preserved thyroid follicles, lined by cuboidal epithelium and containing colloid, were present.

In the 6 animals in which the tissue had been frozen for 2 weeks before autografting, 3 grafts survived for periods of 6 weeks (one animal) and 12 weeks (2 animals). Histologically, in the surviving thyroid tissue the acini were lined by cuboidal cells, and some follicles contained pale colloid (Fig. 1). In the remaining 3 instances, no surviving grafted tissue could be found, but a glial reaction was observed at the graft site.

Comment. Our results indicate that, based on histologic observations, thyroid tissue from the dog can survive freezing and storage for 2 weeks at −196°C and autotransplantation into the cerebral cortex. Although only 50% of the frozen autotransplanted grafts survived, it is of significance that graft survival for a period of 3 months has been demon-

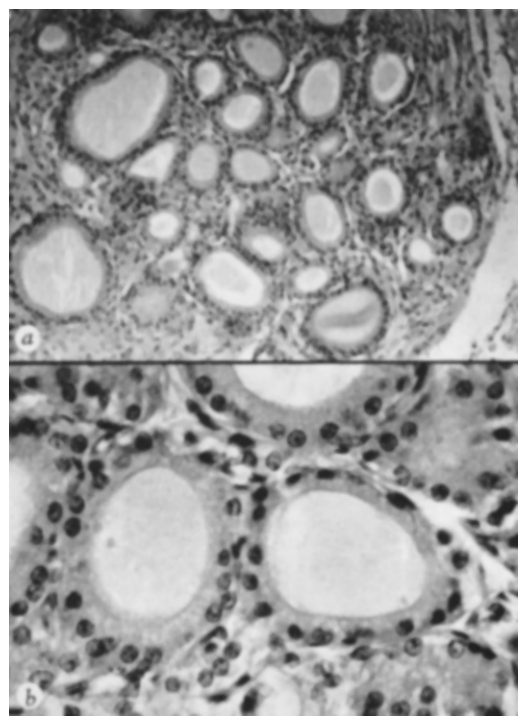

FIG. 1. *a.* Histologic appearance, after 3 mo, of an intracerebral autograft of thyroid tissue after freezing in liquid nitrogen 2 wk before grafting (hematoxylin and eosin; ×85). *b.* High-power view of same graft shown in *a* (hematoxylin and eosin; ×342).

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)

E. P. COHEN,† J. S. INGRAHAM AND D. W. TALMAGE
Department of Medicine, University of Colorado Medical Center, Denver

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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† Special Postdoctoral Fellow, U. S. Public Health Service.