elapsed between biopsy and transplantation of the tissue.

Despite these conditions, the human tumor has grown in seven of the twelve rabbits used. Growth first became apparent toward the end of the third week when the rough edges of the fragments became rounded and their color changed to a pinkish-yellow in contrast to the dull white of pieces that failed to grow. The extension of blood vessels from the iris into the transplant occurred in four of the animals between the thirty-fifth and fortieth days. Vascularization has not occurred to date in three of the animals in which primary growth was observed, but notwithstanding the fragments have continued to increase in size.

The growth rate increased following vascularization, and at the present time the transplants are approximately five times their original size. In two instances the pieces are attached to the cornea, which has apparently been invaded by tumor cells and extension to the outside is imminent. In the remaining instances, the transplants are attached to the iris, and irregular projections of growth may be observed in many planes.

In so far as heterologous transplantation from rabbit to guinea pig is concerned, it has been definitely established that the anterior chamber of the eye affords a means of transplantation of the tissues of one species into the body of another where apparently they may be continued indefinitely. The human cancer has not been carried as far, but since the behavior of the grafts conforms in all respects with those of rabbit tumors in guinea pigs, it seems highly probable that human tissues can also be maintained indefinitely in the foreign host.

The serial transplantation and growth of the tissues of a mature animal of one species in an animal of another species opens up a number of interesting problems. Among them is the question of the specific nature of the tissue ultimately grown in the foreign host. This and other problems are under investigation. Detailed results of this series of experiments will be reported elsewhere.

HARRY S. N. GREENE

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH,
PRINCETON, N. J.

REDUCED BLOOD COAGULATION TIME BY INJECTION OF STEROL EXTRACT OF LIVER

DURING a study of sterols extracted from the liver, a more rapid clotting of the blood was noticed after one of the lipid fractions was injected subcutaneously into a hypophysectomized-depancreatized dog. A preliminary investigation therefore has been made of the effect of this fraction in reducing the clotting time in animals with a hemorrhagic tendency from ligation of the bile duct and from a vitamin K deficiency. Dam[1] and Almquist and Stockstad[2] have described the production of a generalized hemorrhagic tendency in chicks fed a diet deficient in vitamin K and were able to prevent the bleeding tendency by the feeding or injection of extracts derived from alfalfa, hog liver fat, green vegetables and other sources. The reduced prothrombin of rats after bile duct ligation has been elevated by feeding vitamin K, in large amounts or with the addition of bile.[3] Highly potent concentrates of vitamin K which can be injected parenterally have been obtained by Almquist[4] and by Dam and Glavind.[5] They describe the vitamin as a non-sterol, unsaponifiable lipid. Osterberg[6] also described it as a non-sterol, which was alkali-labile and fairly heat-stable.

The substance we employed in this study is a sterol which is fairly stable to alkali and heat. It has been obtained from the livers of dog, lamb and pig by the following method of extraction. One kilogram of ground liver from a freshly slaughtered animal was extracted at 50° C. with 2 liters of 95 per cent. alcohol, which was acidified with 0.3 cc of concentrated HCl, and then re-extracted with 2 liters of 95 per cent. alcohol. The combined filtrates were saponified for 18 hours at 45° C with 50 grams of $Ba(OH)_2$ and 80 grams of NaOH, then held at 0° C for 4 hours before filtering at room temperature. After concentrating the clear dark amber solution to $\frac{1}{2}$ volume with reduced pressure at 40° C it was vigorously shaken with 500 cc of petroleum ether, the alcohol phase was diluted with an equal volume of water and shaken again. Two additional extractions were made with 200 cc portions of petroleum ether. Evaporation of the ether from the combined extracts left about 700 mg of a yellow crystalline material, soluble in about 10 cc of sesame oil at 38° C. Small amounts of this impure material gave a positive Salkowski and Lieberman Burchard reaction but negative Rosenheim and Pettenkoffer tests. Only a few milligrams of precipitate could be obtained by digitonin treatment of 500 mgm of the crude material.

The sterol fractions have been taken up in sesame oil for subcutaneous injection into normal and jaundiced rats and into vitamin K deficient chicks. Dogs have been used for the intravenous injections of the extract, suspended in an emulsion with lecithin in normal saline. For the determination of clotting time blood was drawn in the rat by syringe from the heart

[1] H. Dam, *Nature*, 133: 909, 1934.
[2] H. J. Almquist and E. L. R. Stockstad, *Jour. Biol. Chem.*, 111: 105, 1935.
[3] J. D. Greaves and C. L. A. Schmidt, *Proc. Soc. Exp. Biol. and Med.*, 37: 43, 1937.
[4] H. J. Almquist, *Jour. Biol. Chem.*, 120: 635, 1937.
[5] H. Dam and J. Glavind, *The Lancet*, 1: 707, March 26, 1937; H. Dam, J. Glavind, L. Lewis and E. Tage-Hansen, *Skand. Arch. Physiol.*, 79: 121, 1938.
[6] A. E. Osterberg, *Proc. Staff Meet., Mayo Clinic*, 13: 72, 1938.

or from the femoral vein under light ether anesthesia, in the chick directly from a wing vein into a fine glass capillary tube and in the dog by cardiac or venous puncture. Coagulation time was determined by breaking the capillary tube and confirmed with a drop of blood on a glass plate. Determinations were made on individual animals before injection and at 6 and 24 hours afterward, then daily for several days.

Five to 7 days after bile duct ligation the average clotting time in 30 old male rats weighing 300 grams or more was elevated to 10 minutes. Young male rats weighing 100 to 200 gms did not develop this hemorrhagic deficiency to the same extent, the average clotting time in 30 animals being only 3 minutes in from 6 to 12 days after the operation. Average results after injection of 60 and 70 mgm of the crude sterol extract and 15 mgm of the fraction which was not precipitated by digitonin are shown in the table. Reduction of the clotting time to a normal level occurred within 6 hours and persisted for 24 to 48 hours. Pure cholesterol and cholesterol recrystalized after digitonin precipitation exerted little if any effect as compared to the sesame oil controls. In smaller amounts, 14 to 20 mgms, the crude extract was less effective. Female rats were found to develop the hemorrhagic tendency after bile duct ligation more slowly than the males, showing an average clotting time of 3½ minutes in 6 to 8 days postoperative and 9 minutes in 14 to 22 days. Their response to injection of the various extracts was essentially negative.

TABLE 1

EFFECT OF INJECTED LIVER EXTRACTS ON THE BLOOD CLOTTING TIME (IN MINUTES) OF JAUNDICED RATS AND VITAMIN K DEFICIENT CHICKS

Material injected	Mgm injected	Subject	No. averaged	Hours after injection 0	6	24
Pig liver extr.	60	Old rat	5	7	2	3
Non-dig. ppt. fract. .	15	" "	2	6	2.5	
Cholesterol	10–25	" "	3	13	8	12
Pig liver extr.	70	Young rat	4	4	2.3	3
Cholesterol	25	" "	3	2.7	2.6	3
Lamb liver extr.	20	Chick	8	20	8	3.5
Pig liver extr.	24–60	"	6	14	5	4
Non-dig. ppt. fract. .	15	"	5	15	8	5
Dig. ppt. fract.		"	2	14	3	6
Cholesterol	25	"	8	7	6	4

Subcutaneous injection also was effective in chicks on a vitamin K deficient diet. The average clotting time was 17 minutes for 36 chicks when 14 to 18 days old. After injection of 20 to 60 mgm of the active extract or 15 mgm of the non-digitonin precipitable fraction this was markedly reduced within 6 hours and was decreased further at 24 hours. The effect persisted for several days in some chicks, in others the clotting time increased again after the third day. The small amount of material precipitated by digitonin treatment was very active when tried in 2 chicks, whereas pure cholesterol in 25 mgm doses caused only a slight diminution in the clotting time. We are indebted to Dr. Robert Moore, of the Department of Pathology, for determining that the average prothrombin time was lowered from 10 min. in the controls to 3 min. in the chicks injected with the liver extract.

Normal male rats in a few experiments showed about a 50 per cent. fall in clotting time after administering 15 mgm of the crude extract subcutaneously. In three female dogs, one of which was jaundiced from bile duct ligation, a single intravenous injection of 10 or 15 mgm in lecithin emulsion produced a similar reaction. The clotting time remained at this low level for 48 hours and then gradually increased again. The effect of the intravenous injection was not directly on the blood, since the addition of the extract to freshly drawn dog blood *in vitro* tended to prolong rather than to reduce the coagulation time.

These results indicate that the liver contains a sterol which is active in reducing the blood clotting time of normal and jaundiced dogs and rats. A single intravenous or subcutaneous injection maintains this effect for several days. The extract is similar in action to vitamin K in that it will correct the hemorrhagic tendency in vitamin K deficient chicks when injected subcutaneously. Its sterol nature and resistance to alkali differ from the properties described for vitamin K.[7]

A. L. LICHTMAN
WILLIAM H. CHAMBERS
DEPARTMENT OF PHYSIOLOGY,
CORNELL UNIVERSITY MEDICAL COLLEGE

THE BLOOD VESSELS IN THE CENTRAL NERVOUS SYSTEM OF THE KANGAROO

WISLOCKI and Campbell[1] have recently demonstrated that in the opossum the tissue of the central nervous system is not vascularized by a continuous spongy reticulum of capillaries such as exists in the brains of other mammals, so far as known. Instead, it is supplied by vessels which run in very closely associated pairs, arterial and venous, each member of the pair branching when the other does, and the branches ending as simple loops without any anastomosis. Such an arrangement had formerly been known only in certain lizards (Schöbl[2] and Sterzi[3]) and, in much simpler form, in tailed amphibians (Schöbl,[4] Sterzi[3] and Craigie).[5]

The phylogenetic relationship of the non-anastomosing capillary loops and the freely anastomosing capil-

[7] See references 4, 5 and 6.

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