in normal to excessive amounts in dosages ranging from approximately 20-70 mg/kg (2-5), but no data on tetracycline blood levels were described. However, most of these patients developed a severe metabolic acidosis which was disproportionately severe for the degree of azotemia present. Similarly, patients with impaired renal function given tetracycline developed acidosis, hyperphosphatemia, and azotemia(16). Blood tetracycline levels in these patients were as high as 30 μg/ml. The disproportionate acidosis observed in these studies(2,5,16) does raise the question of protein synthesis inhibition. It is clear that more studies are needed in patients receiving tetracycline to determine whether significant inhibition of protein synthesis occurs.

Summary. Treatment of rats with tetracycline in a dosage of 400 mg/kg results in a syndrome somewhat similar to reported cases in humans of death following intravenous administration of large doses of tetracycline. The rats rapidly developed a severe metabolic acidosis with arterial pH levels frequently decreasing to values below 7.10 units. The acidosis was disproportionately severe for the degree of azotemia present. In addition, marked hyperkalemia frequently developed within 4-8 hours after administration of tetracycline. It is suggested that death resulted from an overwhelming metabolic acidosis or cardiac arrhythmias due to hyperkalemia.

We thank Mr. Joseph Hackett for technical assistance.

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Received April 27, 1967. P.S.E.B.M., 1967, v125.

Mitotic Activity of Rabbit Blood Vessels.* (32312)

T. H. SPAET AND I. LEJNIEKS

Department of Hematology, Medical Division, Montefiore Hospital and Medical Center, New York

Although endothelial cells of blood vessels are functionally active and subject to considerable stress, previous reports have failed to demonstrate convincing evidence of their mitotic activity or turnover in normal, adult animals.(1). Indeed, mitotic activity has not been established in any layer of the normal vascular wall. Most reported observations have been confined to morphological examination of vascular cells; but a single study by Spraragen *et al*(2) noted the absence of labeling in the normal rabbit aorta following

* This study was supported by grant HE 05415-07 from Nat. Heart Inst., USPHS.

administration of tritiated thymidine. The present studies show that labeled cells can be demonstrated throughout the vascular tree of normal, adult rabbits following prolonged intravenous administration of tritiated thymidine.

Methods and materials. Experimental animals were adult New Zealand rabbits of either sex, weighing about 3 kg. Tritiated thymidine was purchased from Schwarz Bio-Research, Inc. It had a specific activity of 15 c/mmole and a concentration of 0.5 mc/ml. The dose of tritiated thymidine given to each animal was 2 mc. This was administered *via* the marginal ear vein, either in undiluted form for pulse labeling or diluted with 100 ml of sterile, isotonic saline for more prolonged injections. After completion of experimental procedures, the animals were sacrificed with intravenous nembutal; pieces of organs were immersed in 10% formalin, and were prepared for histological study by standard methods. Sections were coated with Kodak NTB2 emulsion as described by others(2), were incubated for 7 days at 4°C, were developed according to instructions in Kodak Pamphlet #P-64, and were finally stained with hematoxylin and eosin.

For determination of labeled cells in normal blood vessels, injections of tritiated thymidine were administered to intact rabbits for a 7-hour period, by continuous intravenous drip. Aortic injury was produced by two methods. In each case, operative procedures were performed with aseptic technique, the abdomen was opened by a left lateral incision, the aorta was exposed, and a short segment was dissected free of the surrounding connective tissue. Physical trauma was produced by application of an hemostat, chemical injury by intraluminal application of 1% tetradecyl sulfate and 2% benzyl alcohol (Sotradecol®). One ml of this reagent was slowly infused into the aorta, as blood flow was arrested by proximal application of an hemostat. Occlusion of the aorta was maintained for 1 minute. In animals with injured aortas, tritiated thymidine was given at intervals prior to or subsequent to injury by the pulse labeling method, as designated below. Table I shows the number of animals and schedules employed.

TABLE I. Log of Animals Studied for Effect of Trauma on Aortic Mitotic Activity.

Animal No.	Type of trauma	Day following trauma 1	2	3	4	5	6
1	Clamp	T+					
2	"	T	+				
3	"		T+				
4	"		T+				
5	"		T+				
6	"		T	+			
7	"		T	+			
8	"					T+	
9	"					T	+
10	Sotradecol®	T+					
11	"	T+					
12	"		T+				
13	"		T+				
14	"			T+			
15	"			T+			
16	"				T+		
17	"				T+		

T = Time of tritiated thymidine injection.
+ = Time of sacrifice.

For estimation of labeled cell incidence, counts were performed as follows: In smaller vessels, actual counts were made of total cells throughout sections of different tissues, and the numbers of labeled cells were ascertained simultaneously. In the aorta, total cells of the various layers were estimated by performing multiple counts at random per high power field, and the number of high power fields occupied by sections was determined. Total cells were taken as the count per field times number of fields times number of sections. Spot checking on many slides showed that for a given animal, agreement was about ± 10% from section to section. The sections were all completely scanned a second time for determination of labeled cells. Because of the low incidence of labeled cells, data from all animals were pooled.

Results. Although prolonged searching was required for detection of labeled cells in normal blood vessels, such were ultimately found in the various layers of vessels of all sizes, and in all organs examined. Fig. 1 shows representative examples of labeled vascular cells, and Tables II and III give the incidence of cell labeling based on data from 3 animals. Too few labeled cells were encountered to provide an adequate basis for comparison of labeling frequency among the vari-

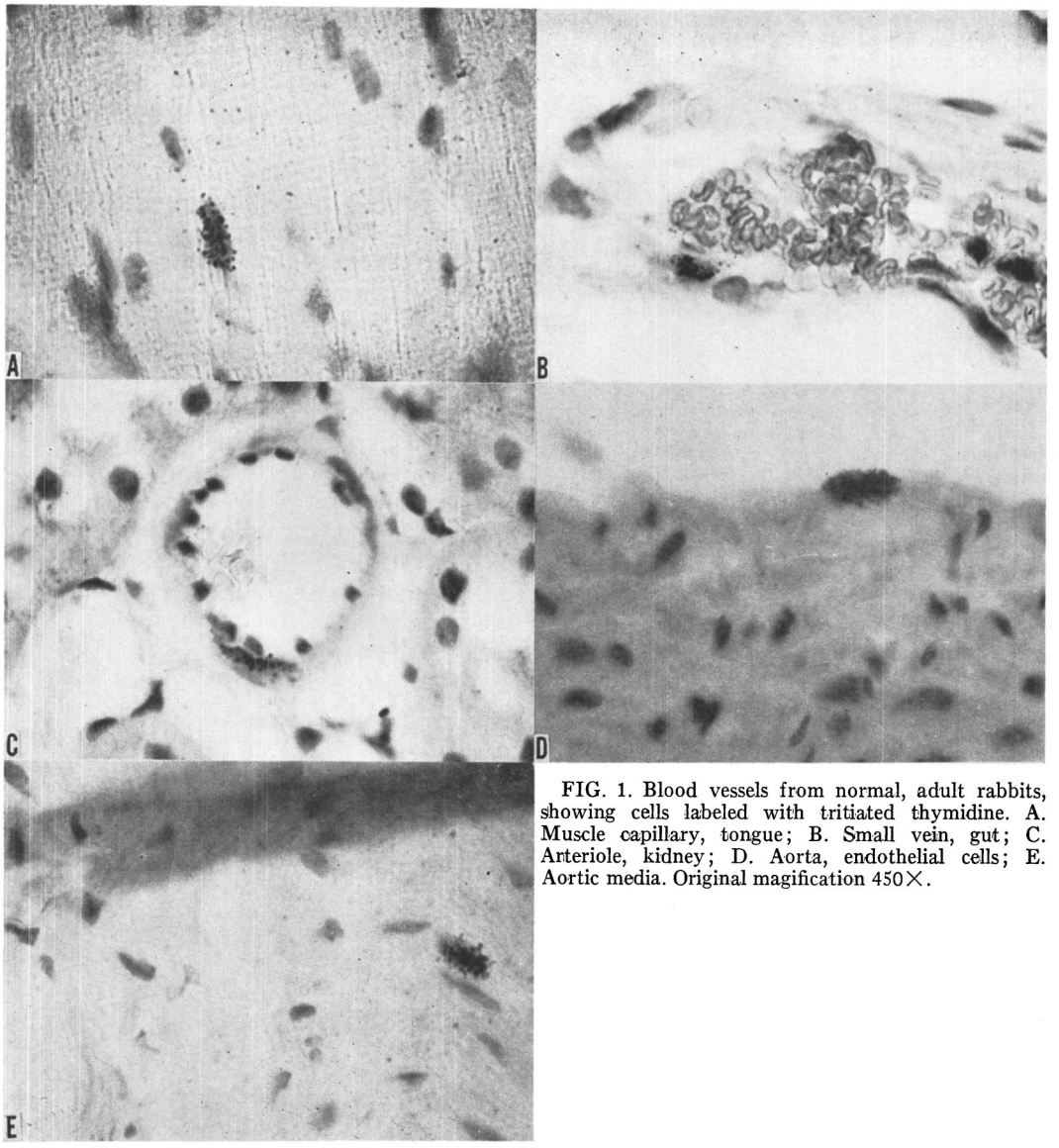

FIG. 1. Blood vessels from normal, adult rabbits, showing cells labeled with tritiated thymidine. A. Muscle capillary, tongue; B. Small vein, gut; C. Arteriole, kidney; D. Aorta, endothelial cells; E. Aortic media. Original magification 450×.

ous components studied, but the results suggest that labeling was more frequent in smaller vessels, and that the aortic media showed the lowest activity.

When the aorta was traumatized by clamping, no increase in labeled cells was evident in animals injected with tritiated thymidine 24 hours after injury. However, after 48 hours a marked increase in mitotic activity was encountered in all layers (Fig. 2). In areas adjacent to the site of trauma, 5% or more of the cells were labeled. However, since the area of injury was focal and irregular, exact quantitation was not possible. Aortas obtained from animals injected as long as 5 days after injury continued to show active proliferative activity of the entire wall. The region of major proliferative activity was within about 1 mm of the injury locus.

In aortas injured by Sotradecol, a similar delay in the appearance of increased labeling was noted, none being evident in 24 hours after administration of the sclerosing agent. In animals studied at 2-4 days, increased mitotic activity appeared. The findings differed from those produced by mechanical

TABLE II. Incidence of Tritiated Thymidine-Labeled Cells in the Normal Rabbit Aorta.

	No. of cells "counted"	Labeled cells/10^4
Intima	15,000	8
Media	680,000	0.3
Adventitia	85,000	6

TABLE III. Incidence of Tritiated Thymidine-Labeled Cells in Normal Rabbit Blood Vessels.

	Intima		Media	
	No. of cells counted	Labeled cells/10^4	No. of cells counted	Labeled cells/10^4
Capillary	10,600	60	—	—
Arteriole	3,000	20	5,900	32
Artery	4,000	25	9,600	16
Venule	1,800	110	3,300	67
Vein	5,500	13	9,600	34

trauma in that labeled cells were primarily in the intimal layer (Fig. 2); very few were seen in the media or adventitia. Also, there was no localization of the mitotic area.

Four animals were given 7-hour injections of tritiated thymidine 2 days prior to production of injury. Two rabbits were then subjected to aortic clamping, the others to Sotradecol injection. After 2 days, the animals were sacrificed, and their vessels were examined for labeling. Although labeled blood leukocytes were numerous, no increased labeling of the aorta was seen in either animal.

Discussion. The ability of the blood vessel to respond to injury with a mitotic response is well known. The presence of reproducing cells in the vessels of normal, adult animals, however, has been questioned(1), although tritiated thymidine labeling in guinea pig endothelium has been detected by an *en face* method (personal communication, Dr. Helen Payling Wright). The present studies demonstrate that labeled cells can be identified in the vessels of adult animals. The incidence of this labeling is such that the vessels can be considered as "growing cell populations" according to the classification of Leblond *et al* (3). It should be noted that the present method compares prolonged infusion of the isotope to pulse labeling of earlier studies (2), favoring an increased incidence of labeled cells.

Several considerations render accurate interpretation of the present data difficult. One in particular is that laboratory rabbits continue to gain weight as they age, even after complete maturity. The degree of vascular growth that accompanies this weight gain cannot be quantitatively assessed. A second problem is the influence of minor trauma, which might lead to proliferative activity in vessels of such superficial organs as the tongue, and vessels in the liver which might be affected by intestinal absorption of toxic agents. Finally, the labeling technique used cannot distinguish between overall turnover of vascular cells, and selective but more active proliferation of local populations. However, if these complications are ignored, it would appear that the smaller vessels replace their cells several times during the adult life of the rabbit; larger vessels have less turnover; and the aortic media is virtually stable.

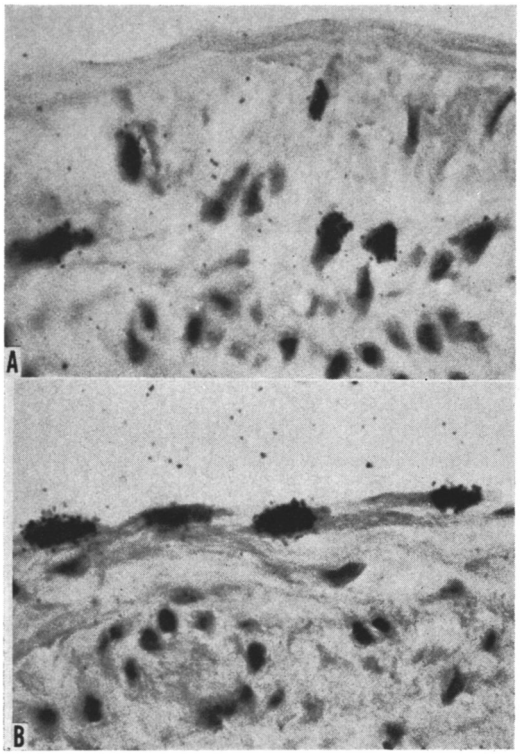

FIG. 2. Labeling with tritiated thymidine by cells of traumatized aortas. A. Near clamped area following physical trauma: B. Following chemical trauma. Original magnification 450×.

It is of interest that the mitotic response to injury is associated with a latent period of about 2 days. Whatever the signal is that denotes a stimulus for vessel cell proliferation, it evidently requires a time-consuming reaction to become effective. The present data suggest that the replacement cells are derived from the blood vessel itself, rather than from a remote pool of proliferating cells. It has been suggested that vascular endothelium may be derived from blood leukocytes(4,5). This hypothesis is not supported by the present observation that labeled cells failed to appear in injured vessels when tritiated thymidine was administered prior to injury, although labeled blood leukocytes were abundant.

Since different types of vascular injury are accompanied by proliferative activity, a practical implication of the present studies is that increased labeling of blood vessels may be a sensitive indicator of damage. Studies are in progress which will use the vessel labeling technique to determine the effects of endotoxin and of experimental thrombocytopenia purpura.

Summary. Rabbit blood vessel cells were labeled with tritiated thymidine. Labeled cells were seen in all layers of all vessels of normal, adult animals, although the incidence was low. Following chemical or physical injury, increased mitotic activity developed after a latent period of 2 days. Injection of isotope prior to vascular injury was unaccompanied by the appearance of labeled cells in the vessel wall.

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Received April 28, 1967. P.S.E.B.M., 1967, v125.

Carnitine Stimulated Transport of the Intermediates of Long Chain Fatty Acid β Oxidation in Liver and Heart Mitochondria.* (32313)

VINCENT W. HOLLIS, JR.,† AND MELVIN BLECHER
Department of Biochemistry, Schools of Medicine and Dentistry, Georgetown University, Washington, D. C.

We have recently reported the chemical synthesis(1) and mitochondrial oxidation(2) of three 1-^{14}C-labeled intermediates of the β oxidation of palmitate, namely, *trans*-2-hexadecenoate, 3-hydroxyhexadecanoate and 3-ketohexadecanoate. In the light of the postulates(3-5) that the carnitine-induced stimulation of the mitochondrial oxidation of long-chain *saturated* fatty acids(6,7) is mediated through the function of O-acyl carnitine as a carrier of saturated fatty acids across mitochondrial membranes, we undertook to examine the possible effects of carnitine on the oxidation of the aforementioned intermediates of palmitate β oxidation by liver and heart muscle mitochondria. Since Norun has reported recently(8) that the enzyme catalyzing the reversible formation of O-palmityl carnitine, namely, palmityl CoA-carnitine palmityl transferase, is elevated in activity in livers of alloxan-diabetic rats, we have also examined the effects of carnitine on the oxidation of the palmitate β oxidation intermediates by liver mitochondria from diabetic rats.

The data presented here demonstrate that carnitine stimulated oxidation not only of palmitate, but also of two of the three β oxidation derivatives of palmitate, by liver and heart muscle mitochondria from normal and diabetic rats. With liver mitochondria, carnitine also increased the accumulation of

* Supported by grant AM-06208 from Nat. Inst. Health, Bethesda, Md.

† Present address: National Inst. of Arthritis & Metab. Dis., Bethesda, Md.