tion(9). Inhibition was seen, which could be in the direction supporting the hypothesis that serotonin has a function in hemostasis; however, the minimum effective concentration of 10^{-2} molar was greater than would be expected physiologically.

The interrelationship of serotonin, histamine, and mast cells(10) and anaphylaxis in which fibrinolytic activity increases(11), stimulated exploration of the possible effect of histamine in the *in vitro* assay. In dilute solutions histamine had little effect; inhibition sufficient to double the control lysis time was seen at a histamine level of 5×10^{-2} M which would be a toxic concentration. Thus, this *in vitro* technic did not demonstrate any role for histamine in the fibrinolytic response observed in anaphylaxis. Oral administration of sulfonylureas has been said to increase the *in vivo* fibrinolytic activity of blood(12), but tolbutamide in high concentration had no effect on the *in vitro* system described here.

Summary. A standardized, urokinase activated, human fibrinogen-plasminogen fibrinolytic system with an arbitrarily controlled lysis time of 13 ± 1 minutes is described. Normal human serum inhibited this fibrinolytic complex. Sera from 8 of 32 patients with various diseases had inhibitor activity significantly above normal. None of these had diseases with abnormal accumulations of fibrin. In ancillary studies epsilon-amino-n-caproic acid inhibited the assay system as, to a lesser degree, did serotonin and histamine; tolbutamide had no significant effect.

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Received June 22, 1962. P.S.E.B.M., 1962, v111.

Regeneration of the Liver in *Triturus viridescens.** (27766)

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The livers of amphibia such as newts(1), frogs(2), and toads(3) were until recently considered to undergo compensatory hypertrophy but not cellular proliferation following partial hepatectomy. Recent work based on observations of mitosis has shown that amphibian liver undergoes at least some degree of hyperplasia after partial removal(4-6). Mitotic figures are few and difficult to find in normal and regenerating newt liver; consequently, tritiated thymidine (H^3-thymidine) and autoradiography were used in this study to detect cells synthesizing deoxyribonucleic acid (DNA). As will be shown, this gives information for study of times and quantitative aspects of cell proliferation.

Materials and methods. Approximately 90 newts obtained from ponds in western Massachusetts, and from commercial suppliers in Massachusetts and Wisconsin, were used. Animals were housed in large aquaria at room temperature (24°C). Versonate,

* Aided by grants from U.S.P.H.S. and by contract from Office of Surgeon General, U. S. Army.

Sodium Diethylenediamine Tetraacetate,† was added to the water, which was changed every 3 days. Two solutions were added to one liter of cold tap water as follows: Solution 1: 20 g $NaHCO_3$ and 20 g Versonate; Solution 2: 10 g $CaCl_2$. Ten ml of each solution was added to each 6 liters of aquaria water. Animals were individually fed small, chopped portions of rat liver each 3 days. They had been separated into those that ate well in the laboratory, so-called "feeders," and those that did not take food. Only feeders were used in these experiments. Tricaine Metanesulfonate, MS-222‡, 1 g per 1,000 ml of tap water was used for anesthesia. Animals were subjected to partial hepatectomy through an anterior midline opening; black silk 6-0 gauge suture was placed about the liver immediately proximal to an incision, and was used to close the abdominal incision. The ratio of liver weight to body weight was determined in 20 non-operated newts. At time of partial hepatectomy, the ratio of liver weight to body weight was also determined. From this, the percentage of partial hepatectomy was calculated. Immediately following operation the animals were returned to a separate aquarium, and the usual feeding and care schedule was followed. To quantitate the number of cells synthesizing deoxyribonucleic acid (DNA), at specified times after operation 200 μc of tritiated thymidine,§ specific activity 1.9 curies per millimole, in a volume of 0.2 ml was injected intraperitoneally using a 1-ml tuberculin syringe and a 21-gauge needle. Animals were sacrificed 3 hours after injection of thymidine on the following postoperative days: 2, 3, 4, 5, 6, 7, 8, 10, 20, 25, 27, 31, 50, 75, 100, 105, 120, and 150. Two partially hepatectomized animals together with one non-operated control were studied at each of these times.

At time of sacrifice body weight and liver weight were determined. Tissues were fixed in 10% neutral buffered formalin for 12 to 20 hours, then processed in the usual way. Paraffin sections were cut at 8 μ, and stripping film (Kodak AR-10), and emulsion (Kodak NTB-3), autoradiographs were prepared. Exposure time of 2 weeks for emulsions and 4 weeks for stripping films was found to be optimal; reducing substances in the liver darkened autoradiographs if exposure times were longer. Autoradiographs were developed in Kodak D-19 developer, rinsed, and fixed in Kodak Acid Fixer(7). Stripping film autoradiographs were stained with hematoxylin and mounted in polyvinylpyrrolidone.‖ Emulsion autoradiographs were stained with hematoxylin and eosin (H & E), and mounted in Permount,¶ a synthetic resin. In autoradiographs the number of H^3-labeled hepatic cell nuclei was counted in 100 consecutive 400× fields, and from determinations of the average number of liver cells in a 400× field, the percentage of H^3-labeled hepatic nuclei was calculated.

Results. 1. *General.* Approximately 10% of animals died within 5 days of partial hepatectomy, due to evisceration of organs or to extensive necrosis in remaining liver. Approximately 10% of animals were discarded because diffuse necrosis of the liver rendered them unusable. The average amount of liver removed at partial hepatectomy was 78% (Fig. 1).

2. *Histology.* Newt livers contained abundant quantities of pigment, apparently melanin, located in Kupffer cells, and they also contained hematopoietic cells in the capsule and in sinusoids. These factors interfered with identification of liver cells at times. Approximately 3 weeks after partial hepatectomy large liver cells with abundant cytoplasm became apparent, and the amount of hepatic melanin pigment was reduced. Mitotic figures were never observed in non-operated newt liver. They were rarely found after partial hepatectomy, and in such small numbers as not to be useful for quantitation of cell proliferation. This contrasted with autoradiographic findings described below.

3. *Restoration of weight.* In normal newts the liver comprised 4.35 ± 0.19% of body weight. The liver weight was restored to nor-

† Fisher Scientific Co., Boston.

‡ Sandoz Pharmaceutical Co., Hanover, N. J.

§ Schwarz BioResearch, Mount Vernon, N. Y.

‖ Antara Chemical Co., New York.

¶ Fisher Scientific Co.

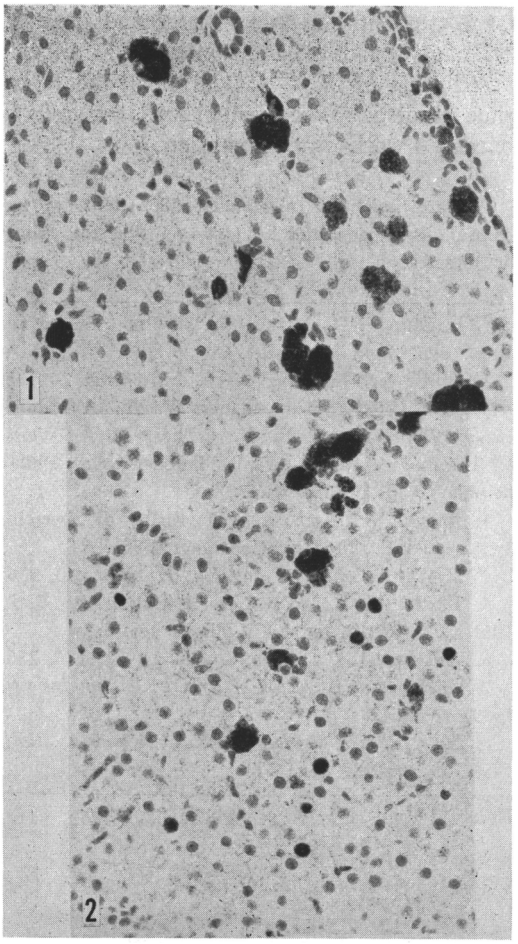

FIG. 1. Emulsion autoradiograph of nonoperated *Triturus viridescens* liver following a single IP injection of 200 μc H^3-thymidine. Note one H^3-labeled hepatic nucleus, lower center; numerous masses of pigment in Kupffer cells; and a bile duct, upper center. Hematoxylin and eosin $\times 200$.

FIG. 2. Emulsion autoradiograph of liver of a *Triturus viridescens* injected IP with 200 μc H^3-thymidine 105 days after partial hepatectomy. Note 6 H^3-labeled hepatic nuclei; the edge of a vein is present in upper left corner. Hematoxylin and eosin $\times 200$.

mal between the third and fourth weeks after partial hepatectomy (Fig. 3).

4. *Autoradiographs.* Occasional autoradiographs were unusable because of the presence of large quantities of reducing substances. In nonoperated newts, the number of H^3-labeled hepatic nuclei following a single injection of H^3-thymidine averaged 0.112% (Fig. 2). Beginning at approximately 5 days after partial hepatectomy the number of liver cell nuclei synthesizing DNA increased to 0.2% and continued to increase up to a peak of 1.9% at 10 days following operation (Fig. 3). At approximately 20 days there was a reduction in number of cells synthesizing DNA, with a second rise to 1.3% at 105 days, suggesting a cyclic response. The number of liver cells utilizing H^3-thymidine was elevated to 0.5% at 150 days after partial hepatectomy, the latest time studied.

A localization of H^3-labeled liver cells was observed in certain areas. Some regions, usually one to three 400$\times$ fields, showed labeling of approximately 10% of cells while other areas, consisting of 10 to 20 400$\times$ fields and many lobules, did not contain H^3-labeled liver cell nuclei. In areas without H^3-labeled liver cell nuclei, Kupffer and hematopoietic cell nuclei showed labeling, indicating that the H^3-thymidine had reached those regions. Although inconstant, there was an occurrence of H^3-labeled liver cells near the capsule and large veins.

Comment. DNA synthesis occurred regularly in newt liver after partial hepatectomy, with a lag period of 5 days between partial hepatectomy and onset of synthesis, similar to that found by Hay and Fishman in newt limb after amputation(8).

Regeneration of the liver in newts appeared to be similar to that in mammals. New cells

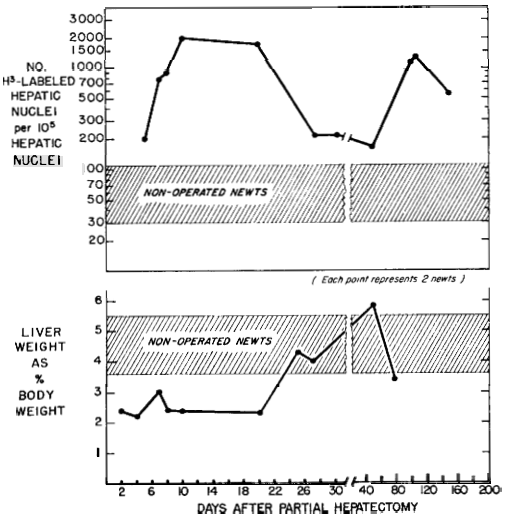

FIG. 3. Uptake of H^3-thymidine by hepatic cell nuclei and restoration of liver weight in *Triturus viridescens* liver at various times following approximately 78% partial hepatectomy.

arose from remaining parenchymal cells. There was a tendency to 2 or more cycles of cell proliferation and there was a tendency for cells in some areas to show more proliferation than other areas. Differences from mammals were a greater lag period between operation and DNA synthesis in amphibia; fewer cells showed DNA synthesis at any one time; and cell proliferation continued for a much longer time after partial hepatectomy.

It was evident from the rarity of mitotic figures as compared with the uptake of H^3-thymidine by liver cell nuclei that mitotic counts alone might give misleading findings regarding the relative importance of hypertrophy and hyperplasia in restoration of amphibian liver. The reasons for this discrepancy are not apparent; amitotic cell division might be a factor.

Summary. Regeneration was studied in the liver of the newt, *Triturus viridescens*, following approximately 78% partial hepatectomy. H^3-thymidine and autoradiographs were used to quantitate DNA synthesis in liver cell nuclei. DNA synthesis began 5 days after partial hepatectomy, reached a peak of 1.9% of liver cells at 10 days, fell to 0.2% at 20 days, rose to a second peak of 1.3% at 105 days, and continued at an elevated level of 0.5% at 150 days, the latest time studied. Liver weight was restored to normal 4 weeks after operation.

We are indebted to Dr. Donald Chalkley, for helpful discussions, and a suggestion that this study be undertaken.

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Received June 28, 1962. P.S.E.B.M., 1962, v111.

Isolation of Dehydroepiandrosterone-C^{14} from Dogs Infused with Cholesterol-4-C^{14} by the Spermatic Artery.* (27767)

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Testosterone and androstenedione are secretory products of the canine testis(1,2). Dehydroepiandrosterone (DHEA), however, has only been isolated from porcine testicular tissue(3) and from rabbit testis slices incubated with acetate-1-C^{14}.† No report of its occurrence in spermatic vein blood has hitherto appeared. Since DHEA and DHEA acetate are converted to testosterone *in vitro*(4) and *in vivo*† it was considered important to establish whether the canine testis secretes this steroid into the blood stream.

Methods and results. The technics for obtaining spermatic vein blood from the left testis and of infusing radioactive compounds by the left spermatic artery of the anesthetized dog have been described(2,5).

Sixty ml of warm saline were saturated with cholesterol-4-C^{14}‡ and infused by the left spermatic artery into normal dogs at a con-

* This work was in part supported by research grants from Nat. Inst. Health, Bethesda, Md. Radioactive cholesterol and steroids were obtained from New England Nuclear Corp., Boston, Mass. The steroids were chromatographed on paper before being used as reference standards.

† Hall, P. F., Sozer, C., Eik-Nes, K., submitted for publication, *Endocrinology*, 1962.

‡ Specific activity: 0.6 μc/mg.