in the renal blood that the flow of this blood into the general circulation causes a decrease of the fibrinolysis time of the euglobulins in the blood obtained for example from aorta or arteria carotis.

In some cases, after pressure on the renal veins or ligation of the ureters, such noticeable activation of fibrinolysis occurs in the euglobulins of the renal blood that during their preparation a proteolytic break-down of fibrinogen takes place, thus showing a lack of coagulation after the addition of calcified thrombin.

This increased fibrinolytic activity may also be observed in the plasma. The plasma in such cases contains fibrinogen and, after recalcination and coagulation, its fibrinolysis time varies from 2.5 to 6 h. In fibrinolytic inactive plasma, no fibrinolysis was observed in 20 h.

Only the RVB euglobulins obtained without stasis and without pressure on the ureters show fibrinolytic activity that is markedly higher than in the arterial blood euglobulins. Euglobulins, from the rabbit venous blood derived from the femoral vein, the marginal auricular vein, the vena cava below the inflow of blood from the renal veins, from the jugular vein and from the bone-marrow blood, do not show any noteworthy fibrinolytic activity compared with the fibrinolytic activity of RAB euglobulins.

The human kidney is similar in this respect to the rabbit kidney. In 5 surgical patients, blood was collected 5 times from the renal veins and cubital veins and 3 times from the renal arteries (Table III).

The results obtained after estimation of euglobulins fibrinolysis time in patients corresponded to those obtained in the experiments with the rabbit blood.

The fibrinolysis time of RVB euglobulins is always shorter than that of RAB and cubital veins euglobulins in man.

Discussion. From the difference in fibrinolytic activity observed between RVB and RAB it may be supposed that the higher fibrinolytic activity of the former is caused by a reduction in fibrinolysis inhibition. This may be a result of a greater excretion of the inhibitors with the urine[10,11] than of plasminogen activator, which is supposed to be carried to the kidney with the blood stream. However, on ligating the ureters an increased fibrinolytic activity of renal blood is also marked and even more noticeable than during blood stasis and takes place in such conditions that a loss of fibrinolysis inhibitors from the blood is practically impossible.

In such conditions an increase in fibrinolytic activity of renal blood can be explained as being the result of an increased concentration of the plasminogen activator produced locally by the kidney and secreted into the blood.

The results indicate the powerful effect of the fibrinolysis activator produced by the kidney which would appear to be the organ controlling the fibrinolysis of the circulating blood.

The presence of UK in human and animal urine appears to be merely a side effect of the much more important function of the kidney; that is, the production of the plasminogen activator and the secretion of it into the circulating blood.

Zusammenfassung. Die Fibrinolysezeit der Euglobuline aus dem Blut der Nierenvenen ist kürzer als die Fibrinolysezeit der Euglobuline aus dem Blut der Nierenarterien des Menschen und Kaninchens. Es wird hieraus gefolgert, dass die Niere Urokinase produziert und somit ein Organ ist, das die Fibrinolyse des kreisenden Blutes kontrolliert.

K. Buluk and M. Furman

Department of General and Experimental Pathology, School of Medicine, Biatystok (Poland), September 25, 1961.

[10] T. Astrup and I. Sterndorff, Scand, J. clin. lab. Invest. *7*, 239 (1955).
[11] G. H. Dillard, J. lab. clin. Med. *36*, 266 (1950).

Effect of Thyroxine on Radioiodine Metabolism by the Thyroid Gland of Goldfish, *Carassius auratus* L., in Fresh and Iodine-Enriched Water

In teleostean fish, the thyroid gland is diffused along the ventral aorta. The basic function of the thyroid is to concentrate iodine and to form from this basic element a characteristic iodoprotein. In general, marine forms, which live in salt-water rich in iodine, accumulate less iodine[1-3]. On the contrary, iodine accumulation is high in fresh-water fish[4-6]. It has also been shown that goldfish, *Carassius auratus* L., behaved differently when kept in fresh-water than they do in iodine-enriched water[7]. The presence of abundant iodine in aquarium water caused the goldfish to take up injected radioiodine less effectively and to excrete more of radioiodine into the water than did the fish maintained in fresh-water which contain very little iodine. This parallels the difference in iodine accumulation and retention in the thyroid between marine and fresh-water fish and suggests that the cause of that difference is associated with the larger stores of iodine which are normally present in sea. The present experiments were carried out to determine if the treatment of the fish with thyroxine brought about any difference.

Material and Methods. Two groups of goldfish were maintained in fresh-water and iodine-enriched water (60 p.p.m. KI solution) in well aerated aquaria in a constant temperature room at 20°C for a week. Each fish was then injected with 40 μc of carrier-free I^{131} intraperitoneally. The solution of I^{131} was made in physiological saline so that it would not interfere with the osmotic balance of the fish. The needle was pushed through the hypaxial muscles of the caudal peduncle to the peritoneal cavity so that any leakage of the radioiodine was prevented when the needle was withdrawn.

At regular intervals starting from 1 h, five fishes were taken out, anesthetized in 1:2000 solution of tricaine methane sulfonate (MS 222), radioactivity of I^{131} was determined on a scaler using a thin window Geiger tube (No. D 34, Nuclear-Chicago Corp.) by the technique described by Swift[8], all the readings were corrected for

[1] J. Leloup, J. Physiol. (Paris) *44*, 284 (1952).
[2] A. Gorbman, S. Lissitzky, R. Michel, and J. Roche, Endocrinology *51*, 311 (1952).
[3] J. Leloup, C. R. Soc. Biol. (Paris) *146*, 1014 (1952).
[4] M. Fontaine, J. Leloup, and M. Olivereau, Arch. Sci. Physiol. *6*, 83 (1952).
[5] G. La Roche, Ann. ACFAS *16*, 134 (1950).
[6] O. Berg and A. Gorbman, Proc. Soc. exp. Biol. Med. *83*, 751 (1953).
[7] P. N. Srivastava, Physiol. Zool. *33*, 277 (1960).
[8] D. R. Swift, J. exp. Biol. *32*, 751 (1955).

decay and regularly checked against a uranium standard. Standard error of the mean was determined for each point. Goldfish has been shown to possess heterotopic thyroid follicles in the head kidney[9], and hence the lower jaw and head kidney region was kept at a constant distance from the thin mica window of the Geiger tube. After the determination of the radio-activity, the fish were returned to their respective aquaria.

Results and Conclusions. Figures 1 and 2 show the results. Previous experiments had shown that in fresh and iodine-enriched water the accumulation of radioiodine in the thyroid reached its peak in 24–48 h[7]. At 24 h, the fishes in fresh-water and iodine-enriched water were subdivided into two sets each, in one of which the fish were injected with 0.4 ml of 0.1% *l*-thyroxine (Nutritional Biochemical Corp.) and the other with 0.4 ml of physiological saline to serve as controls. The results show that because of single thyroxine injection, the fish maintained in fresh-water went on accumulating radioiodine up to 72 h after which the uptake equilibrated. In iodine-enriched water, there was a sharp rise in accumulation up to 72 h, readings, however, equilibrating only after 96 h. Physiological saline injected fish, which served as controls did not show any further uptake after 24 h in fresh-water, whereas in iodine-enriched water a sort of plateau was seen at 24–48–96 h period, maximum accumulation being at 48 h. It is interesting to note that the peak value of thyroxine-injected fish in iodine-enriched water was similar to the peak value of the physiological saline injected fish in fresh-water. Furthermore, the ratio between the peak values of radioiodine accumulation between physiological saline injected and thyroxine injected fish is approximately 1 : 3 in iodine-enriched water and 1 : 4 in fresh-water respectively.

Influence of thyroxine on fish respiration, growth, heart beat, liver glycogen and sexual maturation *etc.* has been extensively reviewed[10]. Its influence on phosphorus metabolism has been shown recently[11]. Injections of thyroxine into the blood stream has shown to inhibit the release of radioiodine. In dog, the release of thyroidal I^{131} was suppressed for 54 h just after 3 h of a single injection of 10 µg *l*-thyroxine[12]. It indicates that, in this respect, the fish thyroid behaves like mammalian thyroid. In iodine-enriched water, at 144 h period, thyroxine injected fish retain 9 times more radioiodine than the controls, whereas in fresh-water, the difference is only 5.5 times. This is because, in iodine-enriched water, the excretion of I^{131} is very fast, a fact which has also been shown in a previous publication[7]. Injection of thyroxine immediately inhibits the release of I^{131}. In fresh-water, the thyroid itself has a greater capacity of retaining iodine, the excretion is slow and hence the difference of iodine accumulation at 144 h between thyroxine-injected and physiological saline in-

[9] W. CHAVIN, J. exp. Zool. *133*, 259 (1956).
[10] G. E. PICKFORD and J. W. ATZ, *The Physiology of the Pituitary Gland of Fishes* (New York Zoological Society, New York 1957).
[11] P. N. SRIVASTAVA, Nature *188*, 512 (1960).
[12] T. YAMADA, S. IINO, and M. A. GREER, Endocrinology *69*, 1 (1961).

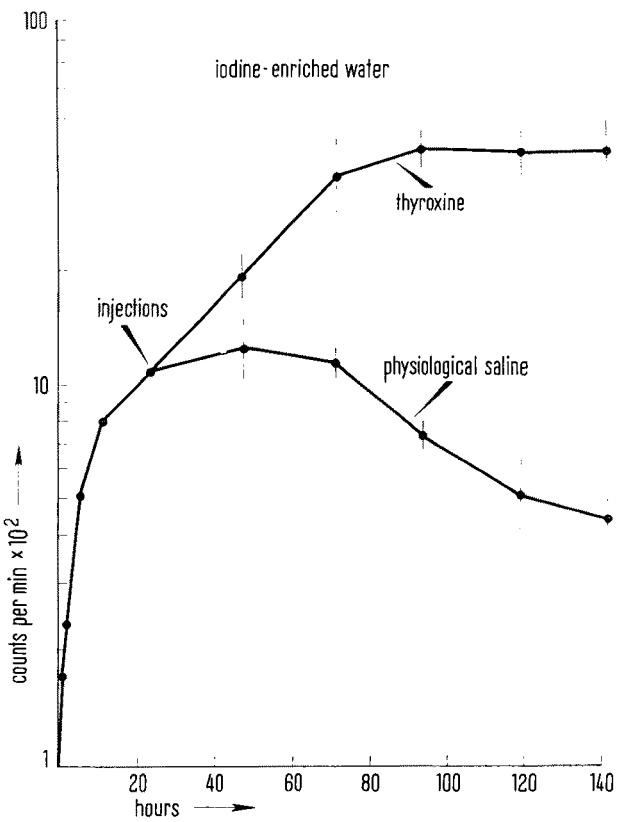

Fig. 1. Arith-log plot showing uptake of radioiodine by the thyroid gland of goldfish in 60 p.p.m. iodine-enriched water.

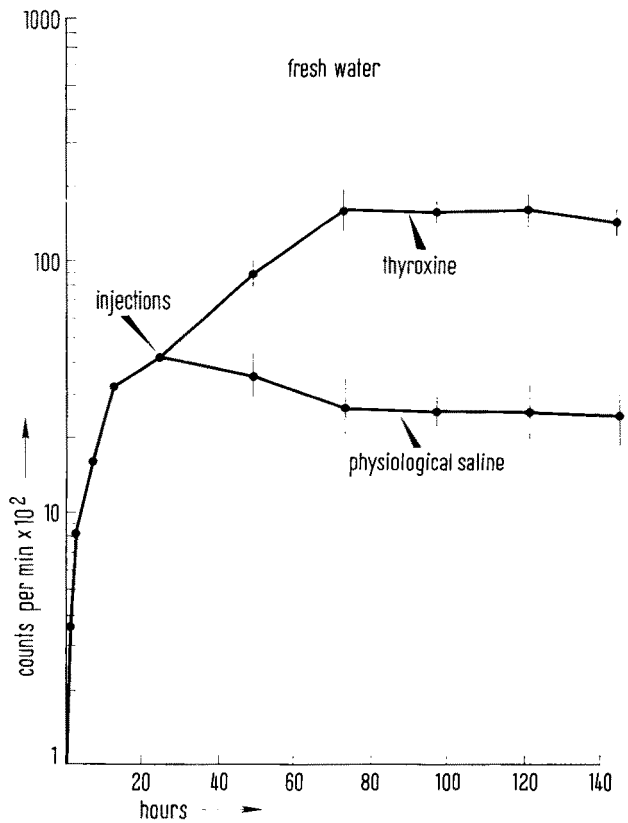

Fig. 2. Arith-log plot showing uptake of radioiodine by the thyroid gland of goldfish in fresh-water.

All points on the graph represent the average of five estimations. At 24 h all the fishes were either injected with *l*-thyroxine or physiological saline. Vertical lines show the standard error of themean, $\sigma_M = /\sqrt{n} = \sigma\sqrt{[\Sigma(v - M)^2]} / n$.

jected fish is also low, as compared to that in iodine-enriched water. The results suggest that thyroxine inhibits the release of I^{131} from the thyroid gland, and in this respect the fish thyroid resembles that of mammals[13].

Zusammenfassung. Die Speicherung und die Abgabe von Jod^{131} nach Injektion von Thyroxin durch die Schilddrüse des Goldfisches hängt vom Jodgehalt des Wassers ab.

P. N. Srivastava

Department of Zoology, University of Rajasthan, Jodhpur (India), September 20, 1961.

[13] The experiments had been carried on in the Zoological Laboratories, Dalhousie University, Halifax, Canada. The author is very grateful to Professor F. R. Hayes for all the helpful suggestions, criticisms, and encouragements. Thanks are also due to the National Research Council of Canada for the award of a post-doctorate fellowship.

Quantitative Determination of Enzymic Activities in Cells and in Extracellular Fluid Aspirated from Human Tumors by Needle Biopsy

In routine work it was observed that the material obtained from some carcinomas (e.g., prostatic or mammary), by means of aspiration biopsy with a fine needle, consists largely of plugs of epithelial cells surrounded by variable amounts of extracellular fluid with occasional admixture of blood. In a previous investigation the epithelial component of aspirated material from prostatic tumors was isolated for quantitative determination of acid phosphatase activity[1]. The present report concerns quantitative evaluation of overall dipeptidase activity in washed epithelial cells and in the extracellular fluid of aspirates from human mammary tumors.

A Luer-lock syringe with a special handle[2] was used together with a stainless steel needle approximately 8 cm long and 0.7 mm in outer diameter (*22* gauge). Use of a thin needle minimizes admixture of blood. The aspirates were taken from 2 or 3 sides of the tumors, without anaesthesia. The material, which was macroscopically free from blood, was used for quantitative determination of enzymic activity (Figure 1).

Figure 2 shows the procedure for separating the extracellular fluid from the cells in order to measure the enzymic activities separately in the cells and in the fluid. Aspirated material from needle biopsy was expressed on to a glass slide and immediately sucked into a breaking pipette, which was then sealed with de Khotinsky cement. The capillary containing the aspirate was cut off and the cut end was also closed with de Khotinsky cement. The material in the sealed capillary was centrifuged at 2000–4000 r.p.m. for 20 min in order to separate the extracellular fluid from the cells. After centrifugation, 0.5 µl of extracellular fluid was obtained in many cases, this being the amount necessary for enzymic assay.

The dry weight and the dipeptidase activity of the cells were thereafter determined as follows. The cells were washed in 0.9% NaCl and resuspended in saline medium. They were then deposited with the aid of a breaking pipette on a disc of millipore filter paper of known weight, which was mounted on a sintered glass filter connected with a pump. The saline medium was removed by suction

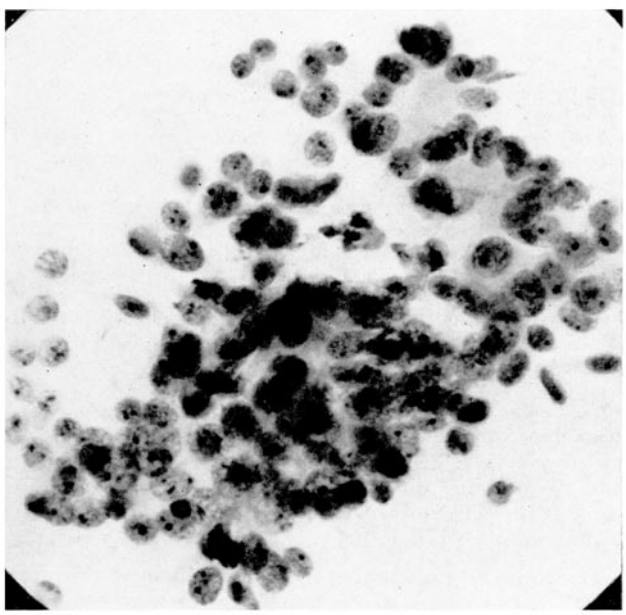

Fig. 1. Plugs of epithelial cells aspirated from mammary carcinoma and spread on a glass slide. Papanicolaou stain.

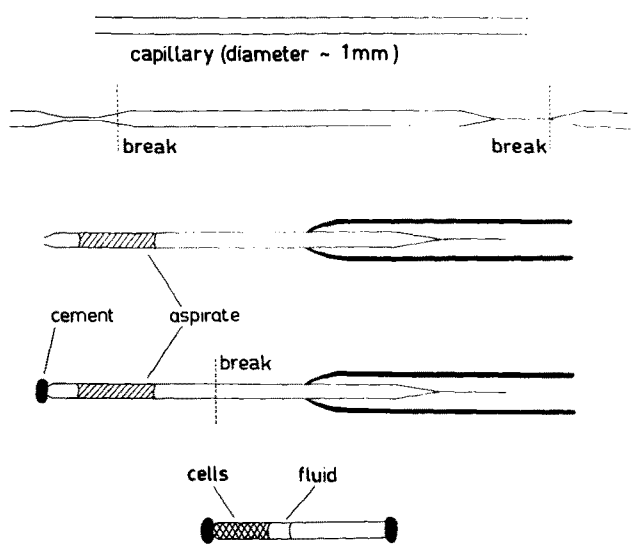

Fig. 2. Method for separating extracellular fluid from cells in material obtained by aspiration biopsy. From a capillary having a diameter of about 1 mm a breaking pipette is pulled. The aspirated material is sucked into the pipette and the open end is sealed with de Khotinsky cement. The part of the capillary containing the aspirate is cut off from the pipette and the cut end is also closed with cement. The extracellular fluid is separated from the cells by centrifugation of the capillary.

[1] P. L. Esposti, B. Estborn, and J. Zajicek, Nature (Lond.) *188*, 663 (1960).

[2] S. Franzén, G. Giertz, and J. Zajicek, Brit. J. Urol. *32*, 193 (1960).