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.

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[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

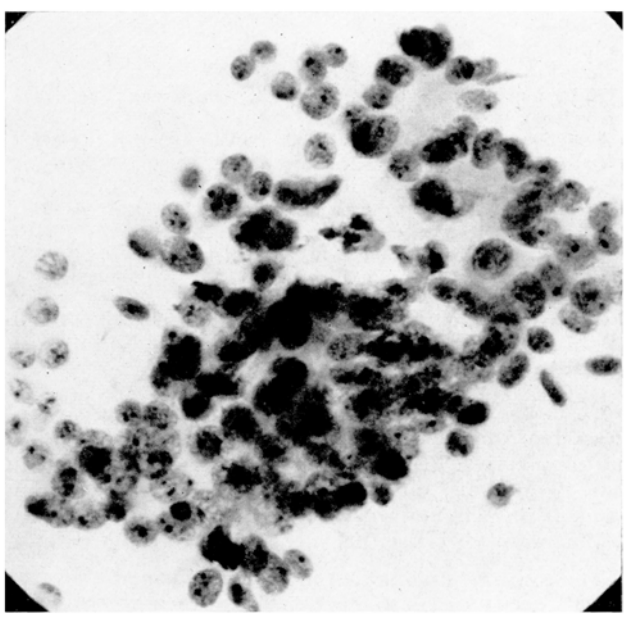

Fig. 1. Plugs of epithelial cells aspirated from mammary carcinoma and spread on a glass slide. Papanicolaou stain.

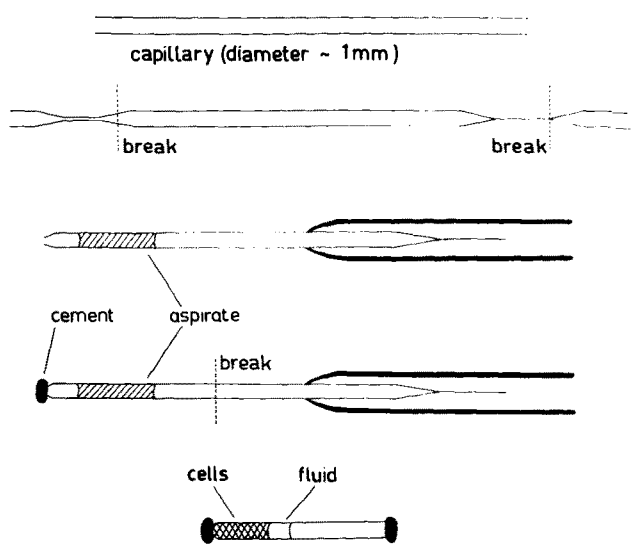

Fig. 2. Method for separating extracellular fluid from cells in material obtained by aspiration biopsy. From a capillary having a diameter of about 1 mm a breaking pipette is pulled. The aspirated material is sucked into the pipette and the open end is sealed with de Khotinsky cement. The part of the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

and the deposited material was air-dried, after which its weight could easily be determined. The filter disc with the cells on it was subsequently submerged in the incubation medium for measurement of enzymic activity. Dipeptidase activity was determined by the method of HOLTER and LINDERSTRÖM-LANG[3] as applied by SYLVÉN and MALMGREN[4].

Table I shows the dipeptidase activity of material aspirated from 3 cases of mammary carcinoma and from 3 cases of mammary fibroadenoma. Measurable dipeptidase activity in the washed cells was of the same order of magnitude in carcinoma as in fibroadenoma. In both groups the dipeptidase activity in the extracellular fluid was much higher than that in the washed cells. Very recently SYLVÉN and BOIS[5] reported high dipeptidase activity in cell-free interstitial fluid from unicentric tumor transplants. On a per volume basis this fluid was up to 100 times more active than other body fluids (peritoneal, etc.).

In Table II the dipeptidase activity in extracellular fluid aspirated from 3 cases of mammary fibroadenoma, 3 of mammary carcinoma and 3 of lymphatic metastases of mammary carcinoma and expressed on volume basis may be compared with the activity in serum, in cystic fluid from 3 cases of mastopathia cystica, and in fluid from lymphoedema of the arm following radical mastectomy (3 cases). It is seen that extracellular fluid from benign and malignant mammary tumors contained about 50 times more dipeptidase activity than did serum, cyst or oedema fluid.

Tab. I. Dipeptidase activity in cells and in extracellular fluid expressed on D.W. basis.

	Cases: No. 1	No. 2	No. 3
Mammary fibroadenoma:			
Cells	5.0	1.1	2.2
Extracellular fluid	17.0	19.0	8.0
Mammary carcinoma:			
Cells	2.6	0.4	4.9
Extracellular fluid	11.0	24.0	37.0

Dipeptidase activity is expressed in µl 0.1 *M* NaOH solution per 100 µg air dried material. Substrate 0.1 *M* alanyglycine. The incubation time 1 h at 37° (pH 7.8).

Tab. II. Dipeptidase activity in body fluids expressed on volume basis

	Cases: No. 1	No. 2	No. 3
Extracellular fluid from:			
Mammary fibroadenoma	10.8	7.4	14.8
Mammary carcinoma	18.8	11.2	21.8
Carcinomatous lymph nodes	18.8	19.9	17.4
Serum	0.40	0.22	0.24
Mammary cyst fluid	0.23	0.42	0.36
Lymphedema fluid	<0.05	<0.05	<0.05

Dipeptidase activity is expressed in µl *M* NaOH per 1 h per µl body fluid. Substrate 0.1 *M* alanylglycine. The incubation time 1 h at 37° (pH 7.8).

These observations do not indicate the origin of the dipeptidase activity found in the extracellular fluid of primary and metastatic mammary tumors (Table II). Histochemical studies have shown high aminopeptidase activity in the stromal elements[6–8] as a reaction to tumor growth[9]. It is possible that the high dipeptidase activity in extracellular fluid of mammary tumors is due to release of the enzymes from the tumor cells as well as from the cells of the stroma compartment to the surrounding medium, after which the enzymes, together with the extracellular fluid and with cancer cells, may be conveyed through lymphatic channels into regional lymph glands. There is some evidence that the successful growth of neoplastic cells can in part be explained by superior ability of such cells to capture from the environment, and to concentrate intracellularly, the free amino-acids necessary for protein synthesis[9]. High concentration of proteolytic enzymes, including dipeptidases, in the extracellular fluid and possibly also in the lymphatic channels, presumably constitutes a factor enhancing the neoplastic growth.

A more detailed account of the method we have used will be published later, together with a discussion of the results hitherto obtained.

Zusammenfassung: Es wird eine Punktionsmethode zur quantitativen Bestimmung der Enzymaktivität in Zellen und interstizieller Flüssigkeit menschlicher Tumoren beschrieben. Die in den Zellen und der interstiziellen Flüssigkeit von Mammacarcinomen und -fibroadenomen gemessene Dipeptidaseaktivität wurde mit derjenigen verschiedener Körperflüssigkeiten verglichen.

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Tryptophan im aktiven Zentrum von Trypsin und Chymotrypsin

Eine Reihe von Hydrolasen wird durch Diisopropylfluorphosphat (DFP) blockiert. Nach salzsaurer Hydrolyse ist der DIP-Rest an ein Seryl gebunden, das in einer Aminosäurensequenz liegt, die für neun Esterasen in verschiedener Ausdehnung identisch ist. OOSTERBAAN et al.[1] ziehen eine enzymatische Spaltung der Säurehydrolyse vor, da sie weniger Gefahren von Transaminierungen bzw. Transacylierungen bietet, fanden aber den Phosphor am gleichen Serin. Allen auf diesem Gebiete bisher eingesetzten Analysenmethoden ist jedoch gemeinsam, dass sie zum Verlust des Tryptophans führen. HARTLEY gab nun in Moskau[2] einen grossen Teil der Aminosäurensequenz des Chymotrypsins bekannt, der zwar kein Histidin, wohl aber zwei der sieben Tryptophane enthält,

[1] R. A. OOSTERBAAN et al., Biochim. biophys. Acta *27*, 549 (1958), und V. Int. Congr. Biochem. Moskau *4*, 130 (1961).
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