

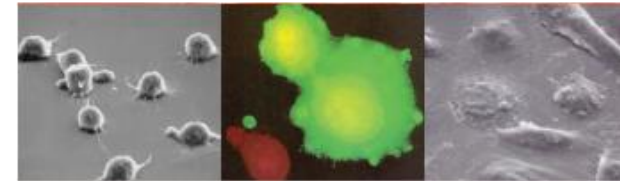
The initial cancer biology research studies, as shared with Henryk Taper:

Finding that a deficiency in alkaline and acid nuclease activities in tissues make them prone to carcinogenesis.

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1. Henryk TAPER or the passion for cancer research.



Dear Colleagues and Friends:

I hold at heart to come here to bear witness to the common and shared passion that I lived with my late friend Henryk Taper, especially between 1965 and 1980.

We have been beating together with common goal efforts: to search about the origin of cancer as we shared the similar background; both of us trained Clinician Internists, but also Morphologists and Biologists.

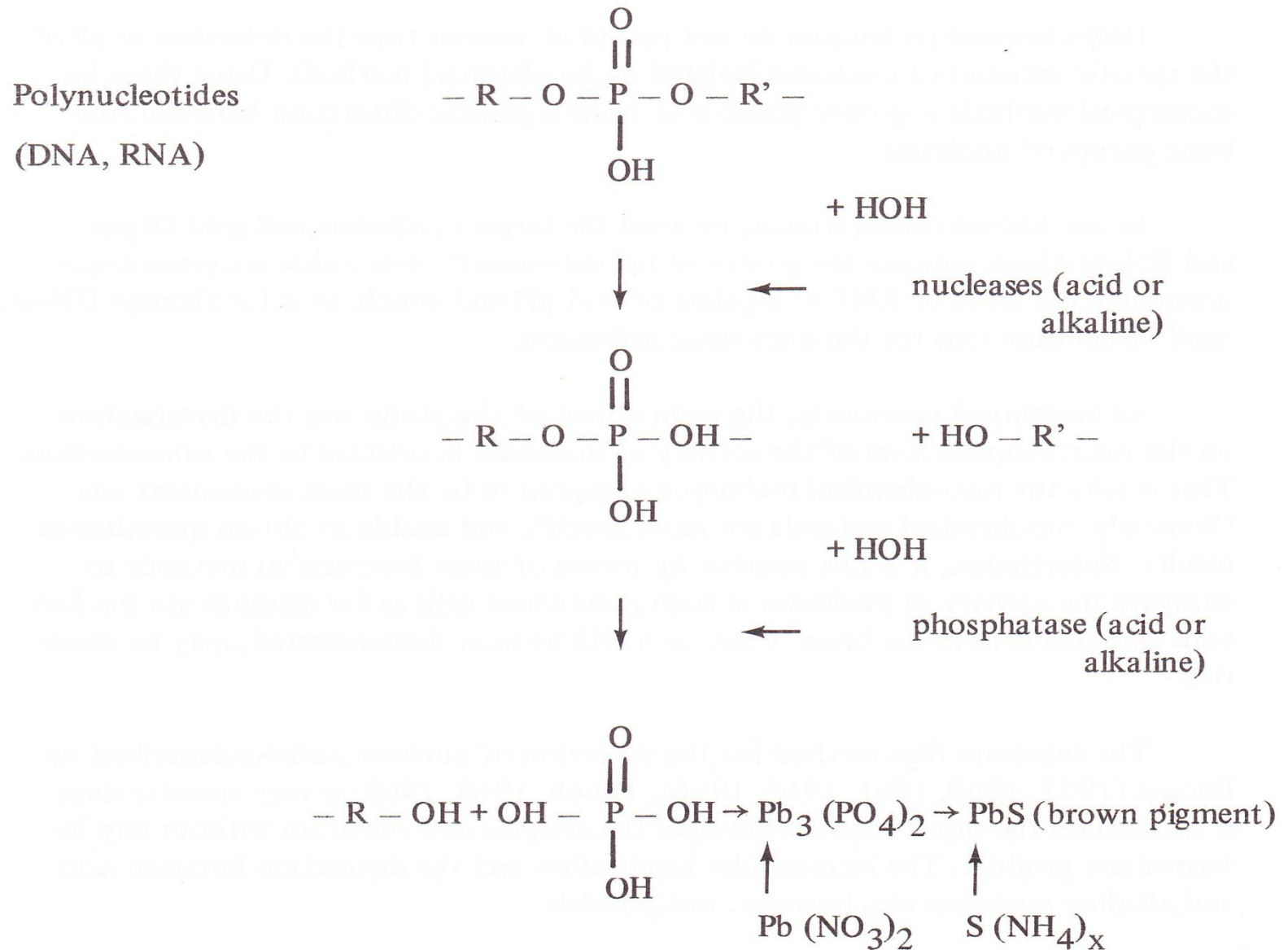
As you probably remember more than 50 years ago, the medical scientific world got shaken by two big discoveries: (1) during the 1920s, the discovery of insulin by Banting and Best and from this research accomplishment (2) the team of Professor Christian de Duve, of our University, studying glucose metabolism in the liver and, particularly, of the acid phosphatases; those findings ultimately made de Duve to discover the lysosome concept.

Another big accomplishment was Watson and Crick describing the double helix of DNA.

This last finding made more research studies to be oriented toward heredity. This is also why, at first, in 1959, R. Daoust and A. Cantero, at the Institut Notre-Dame of the Université de Montréal, applied gelatine films containing DNA over intact tissues and tumours and demonstrated that the malignant tumours have no nuclease activities.

In 1968, Henryk Taper found a way to evidence histochemically alkaline and acid DNases and RNases on slices of living tissues (1968) (Projection **Slide / Dia 1**) and as soon as that finding, he had to defend against the incredible biochemists (Slides / Dia 2, 3, 4)

TABLE I : MAIN FEATURES OF THE REACTION FOR HISTOCHEMICAL DETECTION OF NUCLEASES



The first critique was: Does the freezing and fixation maintain tissues alive? And Henryk showed they were...

The second critique: Does the pH difference modify the membrane charge and the capture of metals? Henryk showed it too.

TABLE 11 ACTIVITY OF ACID DNASE IN NORMAL RAT LIVER MEASURED BY BIOCHEMICAL METHOD AT DIFFERENT STAGES OF THE HISTOCHEMICAL PROCEDURE

STAGES OF THE HISTOCHEMICAL PROCEDURE

STAGE OF THE HISTOCHEMICAL PROCEDURE	ACID DNASE ACTIVITY				NUMBER OF MEASUREMENTS
	UNITS/G OF TISSUE	PERCENTAGE OF PRESERVED ACTIVITY	UNITS/G OF NITROGEN	PERCENTAGE OF PRESERVED ACTIVITY	
NORMAL LIVER	1.11 +/- 0.04 (1)	100	29.7 +/- 2.9	100	9
LIVER SLIDES AFTER CRYOSTAT	1.14 +/- 0.12	100	27.8 +/- 3.5	94	4
LIVER SLIDES AFTER CRYOSTAT + FIXATION	1.71 +/- 0.15	62.5	28.7 +/- 4.7	97	8
LIVER SLIDES AFTER CRYOSTAT + FIXATION + 2 mM LEAD NITRATE IN INCUBATION MEDIUM	0.63 +/- 0.69	55.5	26.5 +/- 5.7	89.5	4

(1) +/- NUMBERS INDICATE STANDARD ERROR OF MEAN (S.E)

TABLE III

ACTIVITY OF ALKALINE DNASE IN NORMAL RAT LIVER MEASURED BY BIOCHEMICAL METHOD AT DIFFERENT STAGES OF THE HISTOCHEMICAL PROCEDURE

STAGE OF THE HISTOCHEMICAL PROCEDURE	ALKALINE DNASE ACTIVITY				NUMBER OF MEASUREMENTS
	UNITS/G OF TISSUE	PERCENTAGE OF PRESERV. ACTIVITY	UNITS /G OF NITROGEN	PERCENTAGE OF PRESERV. ACTIVITY	
NORMAL LIVER	0.308 \pm 0.108 ⁽¹⁾	100	6.96 \pm 1.4	100	4
LIVER SLIDES AFTER CRYOSTAT	0.423 \pm 0.121	137	9.4 \pm 2.5	135	4
NORMAL LIVER	0.445 \pm 0.074	100	10.1 \pm 1.4	100	8
LIVER SLIDES AFTER CRYOSTAT + FIXATION	0.307 \pm 0.048	69	11.7 \pm 2.6	116	8
NORMAL LIVER	0.583 \pm 0.096	100	13.1 \pm 1.1	100	4
LIVER SLIDES AFTER CRYOSTAT, FIXATION + 2MM LEAD NITRATE IN INCUBATION MEDIUM	0.294 \pm 0.046	52	12.0 \pm 3.24	91.5	4

(1) \pm NUMBERS INDICATE STANDARD ERROR OF MEAN (S.E.)

The third critique: Does the presence of metals alter the histochemical reactions? Yes, but not as it concerns the lead nitrate and in a series of experiments to verify the question, in absence of lead nitrate, no staining was possible.

TABLE IV THE INFLUENCE OF DIFFERENT CHEMICAL COMPOUNDS ON THE HISTOCHEMICAL ACTIVITY OF ALKALINE DNASE AND PHOSPHATASE IN NORMAL RAT DUODENUM, KIDNEY AND IN COUJARD'S SLIDES.

CHEMICAL COMPOUND	CONCENTRATION	ACTIVITY OF ALKALINE					
		DNASE			PHOSPHATASE		
		DUODENUM	KIDNEY	COUJARD'S SLIDES	DUODENUM	KIDNEY	COUJARD'S SLIDES
SODIUM ARSENATE	0,001 M	- (3)	-(2)	- (1)	- (3)	-(2)	- (1)
POTASSIUM SELENIDE	0,02 M	- (3)	-(2)	- (1)	- (3)	-(2)	- (1)
N-BROMOSUCCINIMIDE	0,01 M	- (3)	-(2)	- (1)	- (3)	-(2)	- (1)
ZINC CHLORIDE	0,03 M	- (3)	-(1)	- (1)	+ (3)	+(1)	+ (1)
CUPRIC SULFATE	0,01 M	- (3)	-(2)	- (1)	+ (2)	+(2)	+ (1)
POTASSIUM FLUORIDE	0,02 M	+/- (3)	+/- (3)	- (1)	+ (3)	+(3)	+ (1)
DISODIUM TETRABORATE	0,02 M	+/- (3)	+/- (2)	- (1)	+ (3)	+(2)	+ (1)

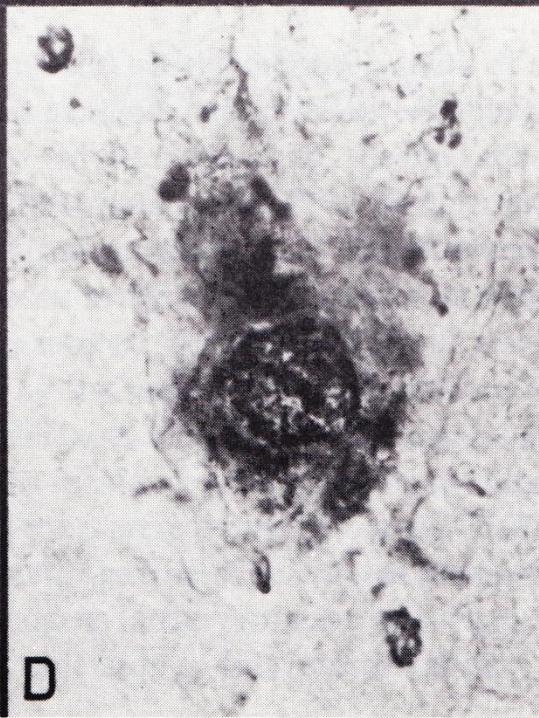
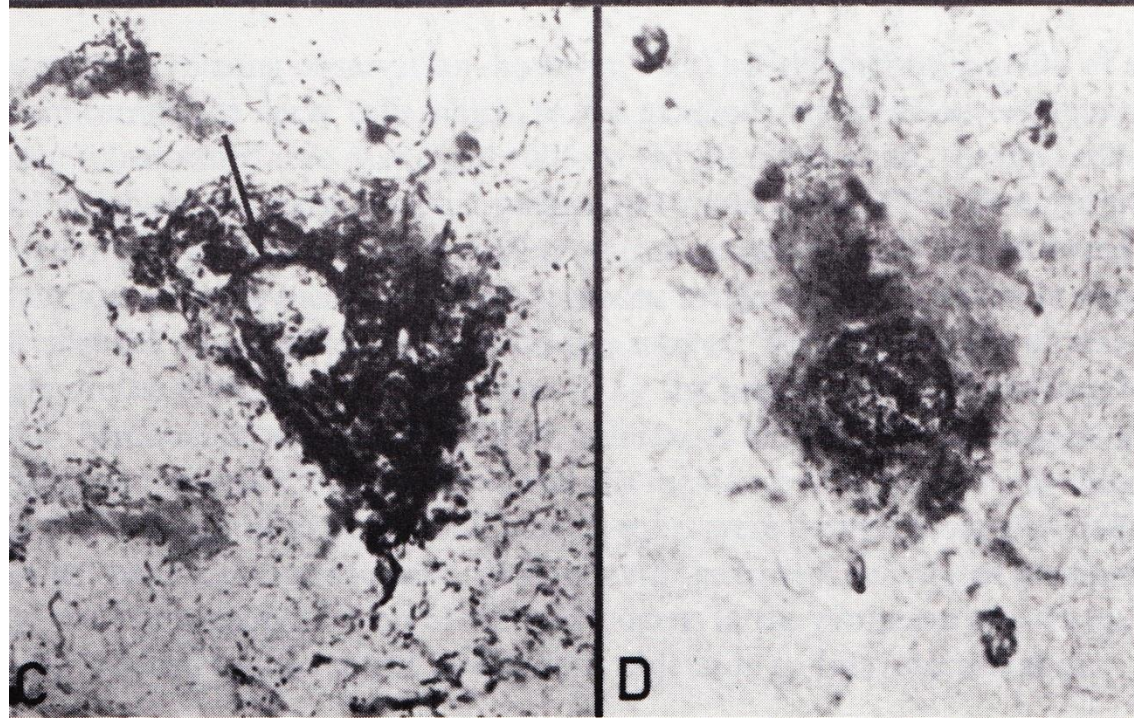
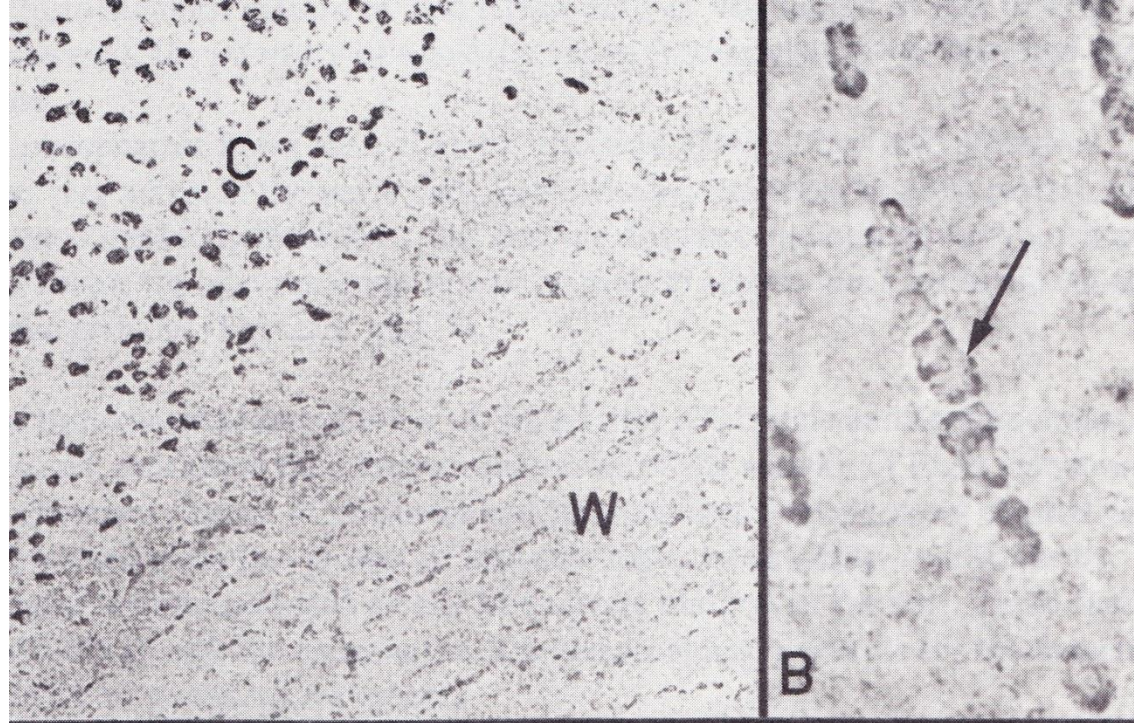
- = ABSENCE OF HISTOCHEMICAL STAINING

+ = POSITIVE HISTOCHEMICAL STAINING

+/- = LOCAL INHIBITION OF HISTOCHEMICAL REACTION (IN NUCLEI OR IN CYTOPLASM)

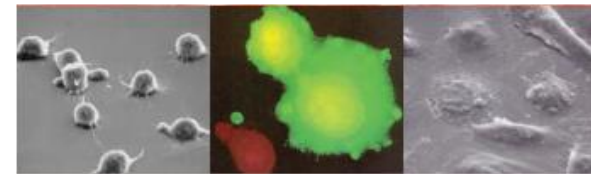
NUMBERS IN PARENTHESIS INDICATE NUMBER OF PERFORMED TESTS.

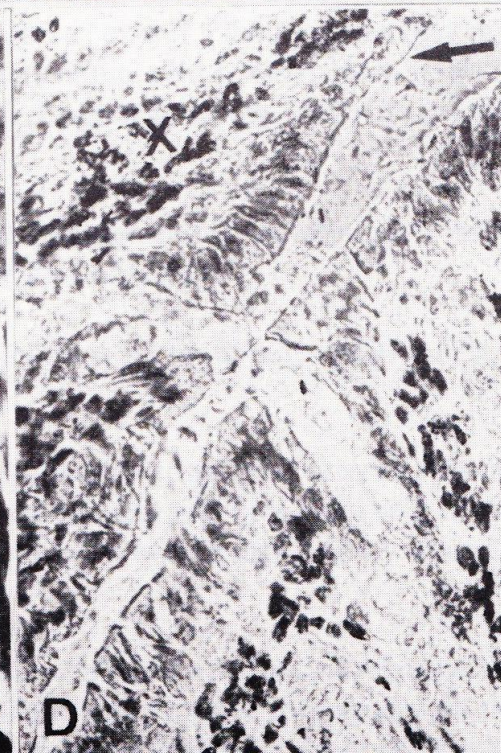
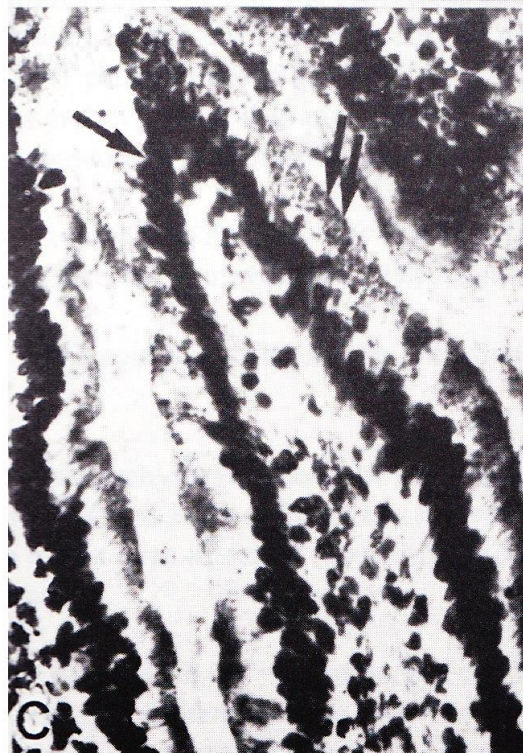
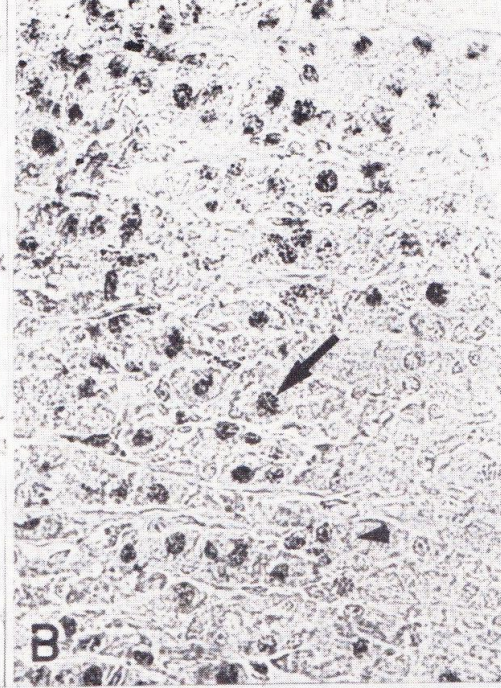
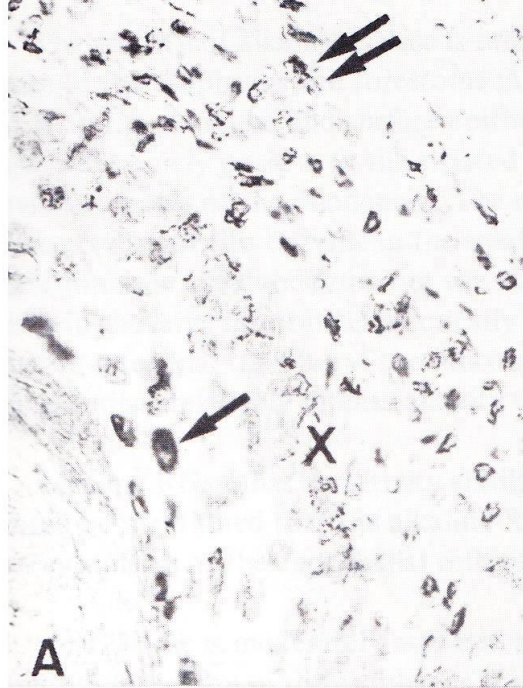
As soon as the methodology was refined, Henryk practised to detect alkaline and acid DNases and RNases in the central nervous system (**Dia 5**): Numerous sites of acid phosphatases were detected in the cytoplasm of neurons and those correspond to numerous lysosomes. Similarly much was also found in nuclei and cytoplasm of the same cells while the activities were very weak in neuroglial cells of the white matter.

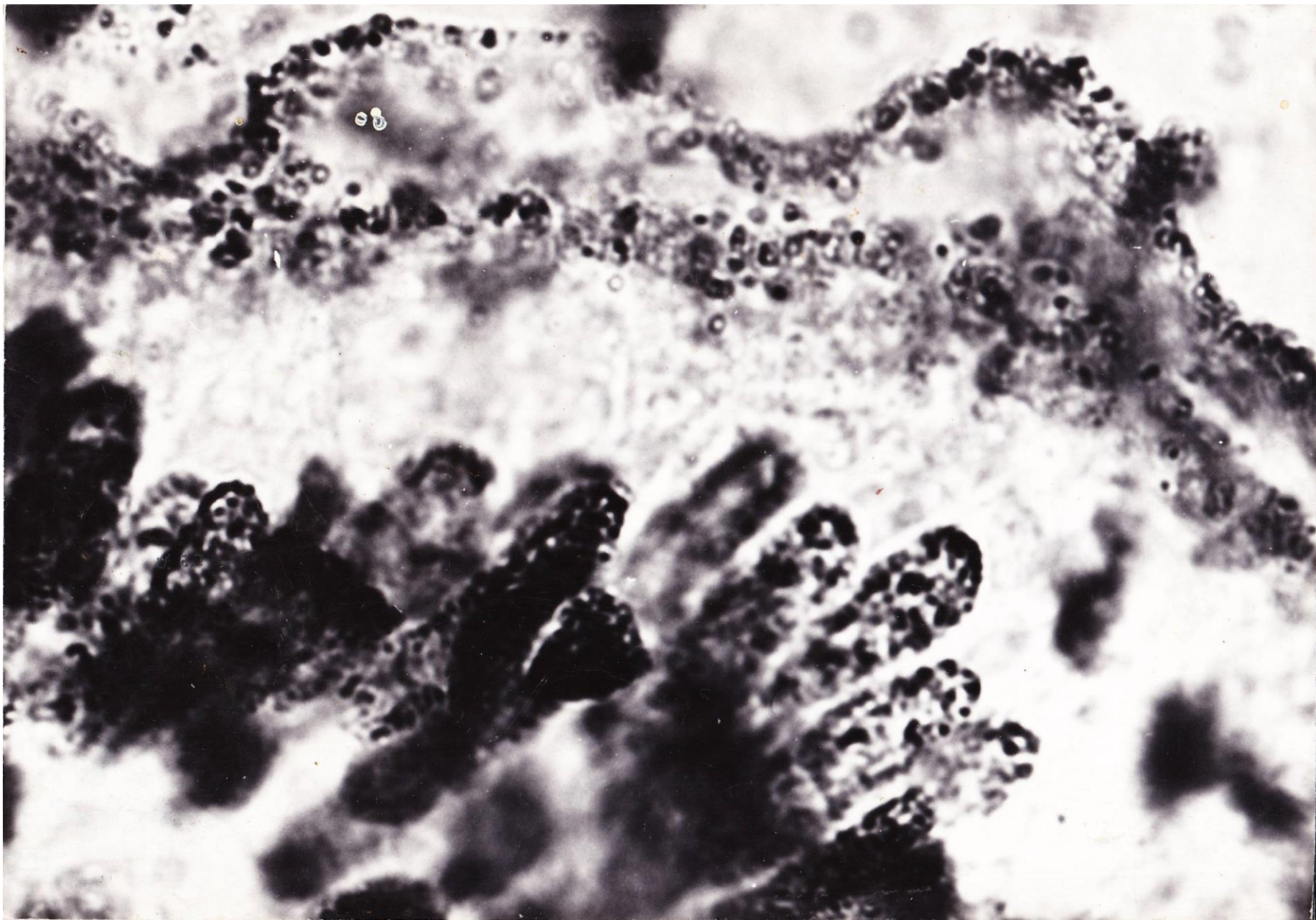


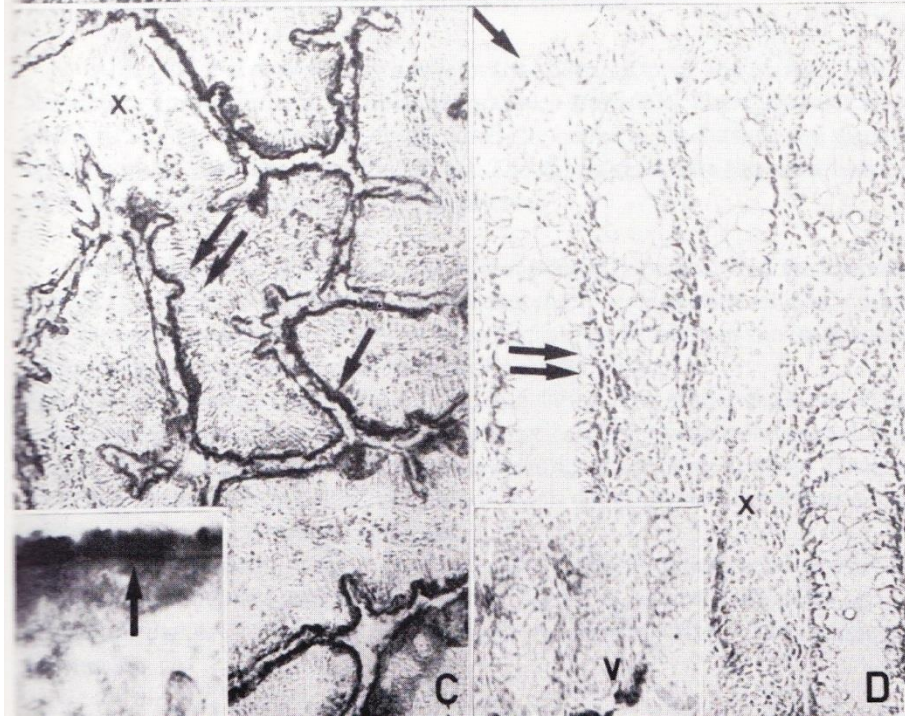
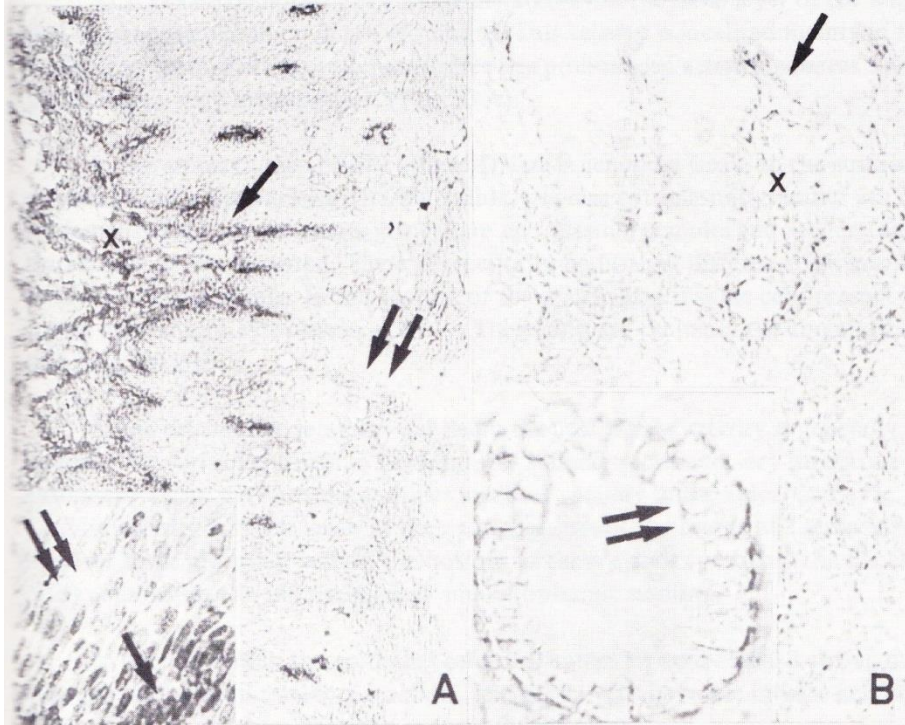
We proposed and concluded that the nuclease activity in tissues is inversely proportional to carcinogenesis and tried to verify this proposition in the digestive system.

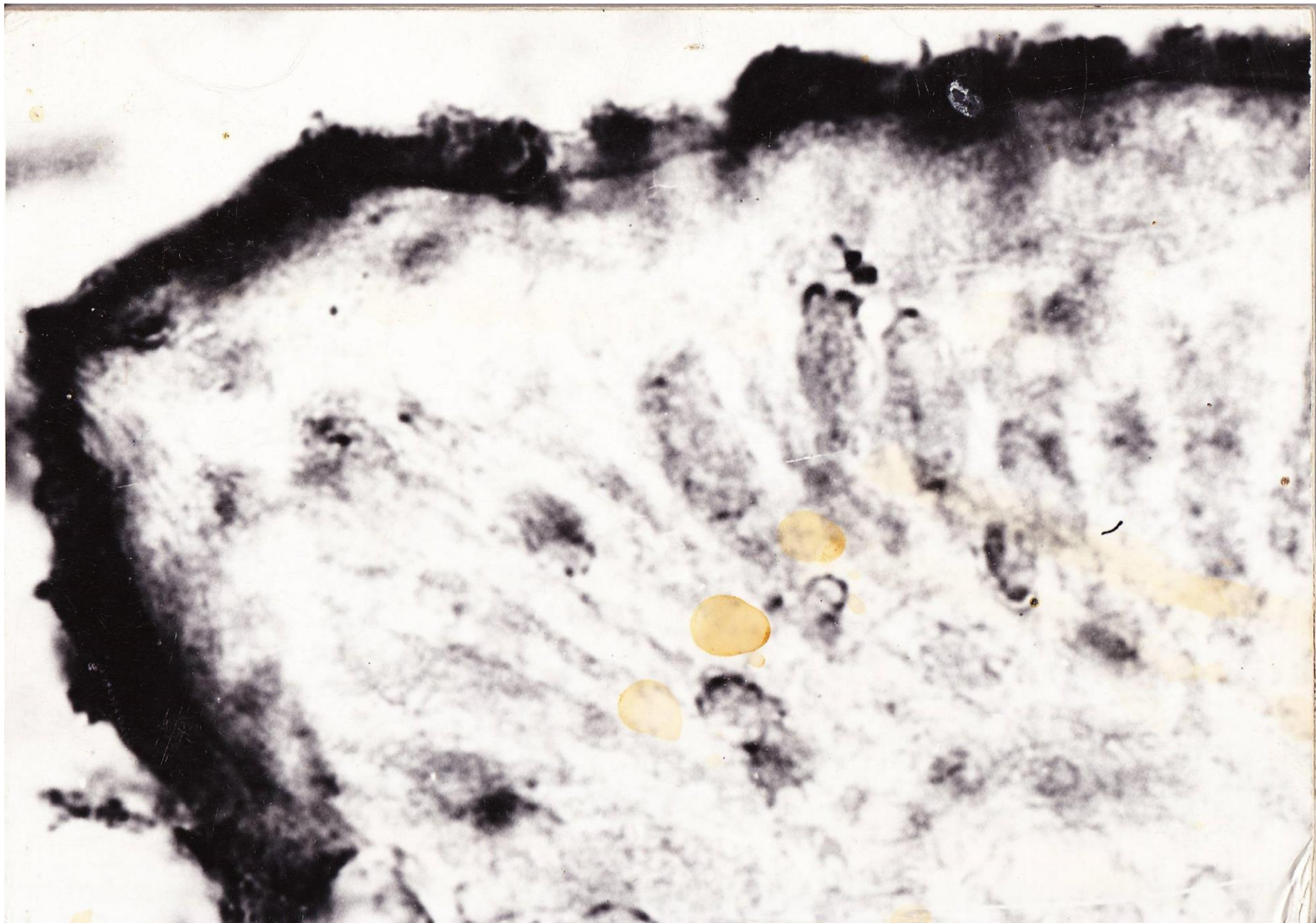
2. The intensity of intracellular or intrinsic alkaline and acid nuclease activities is inversely proportional with the incidence of carcinogenesis in human or animal organs.











We then found that in human and animals a greater nuclease activity throughout the lining of the small intestine even though this organ reaches a surface of about 2 m² in which one rarely found malignant tumours.

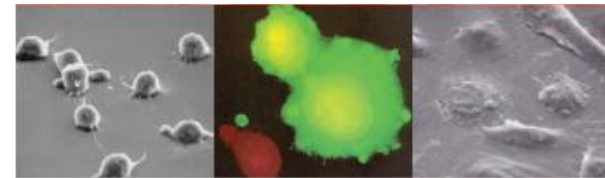
Slide 7 demonstrates alkaline nuclease activity and **Slide 8** acid nuclease activity in the oesophagus, where some basal cells of the epithelium display a positive reactivity. The stomach epithelium shows little activities but the chief cells. At the highest magnification, one can detect that the alkaline phosphatase activity is principally located along the brush border of the small intestine and thus insures an extracellular protection; for the acid phosphatase activity it is clearly demarcated in the lysosomes and other intranuclear sites (**Slides/ Dia 9 and 10**)

TABLE XXIV THE ACTIVITY OF NUCLEASES IN THE MUCOSAL EPITHELIUM OF HUMAN GASTRO-INTESTINAL TRACT COMPARED TO THE INCIDENCE OF MALIGNANT TUMORS DERIVED FROM THIS EPITHELIUM.

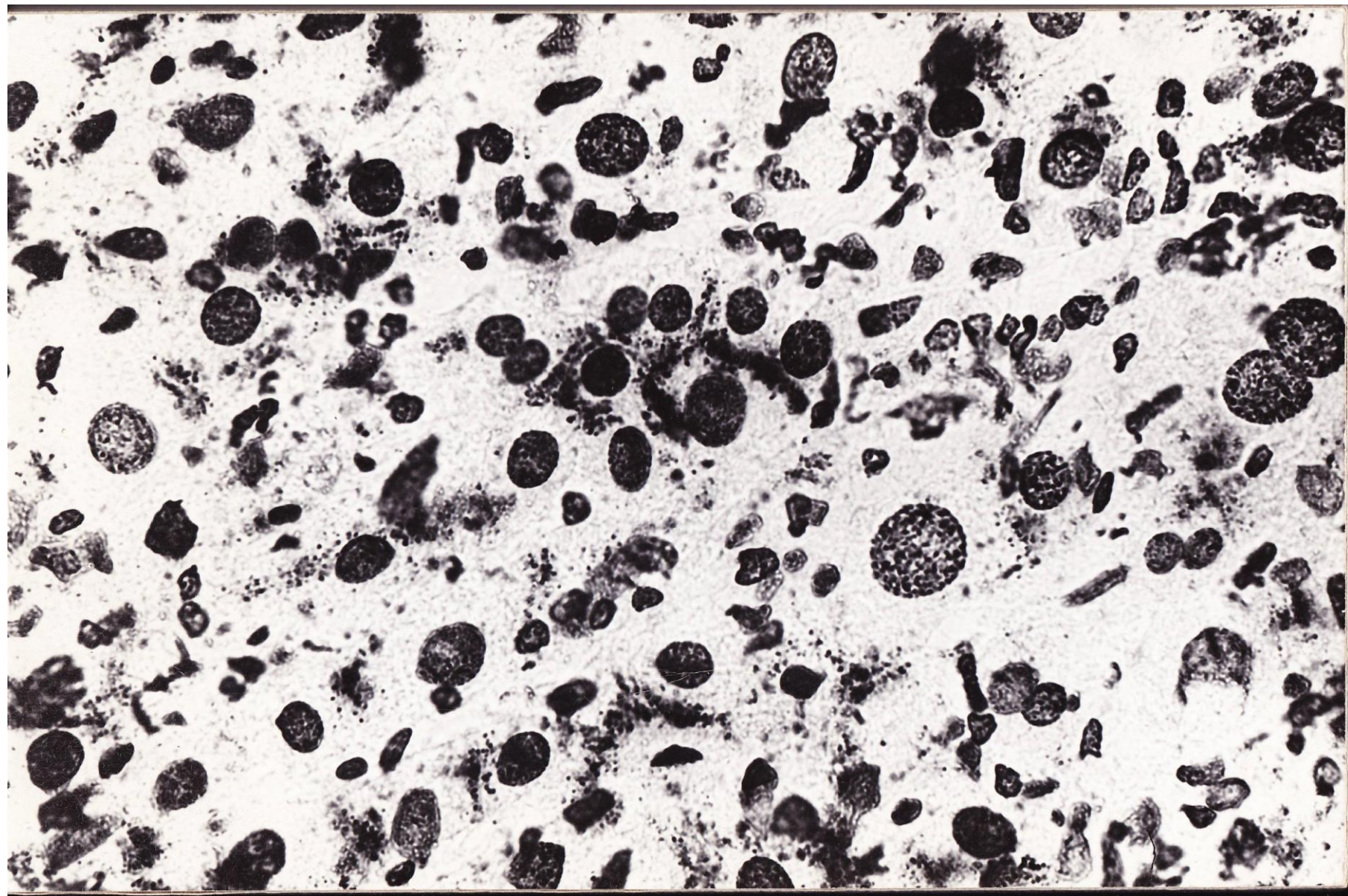
	Esophagus	Stomach	Small intestine	Large intestine
Activity of alkaline nucleases (a)	++	+	+++ ^(b)	+
Activity of acid nucleases	++	++ ^(c)	+++	+
Percentage of epithelial malignant tumors (carcinomas) according to american statistics (Dorn and Cutler, 1955)	6,9	28	0,9	64,2

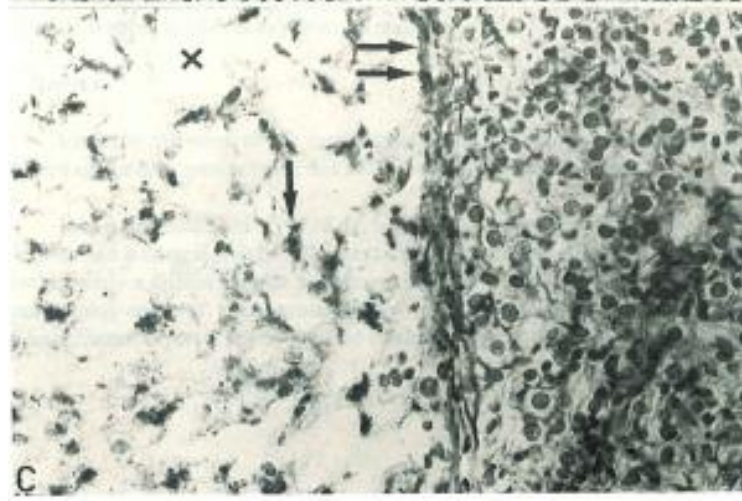
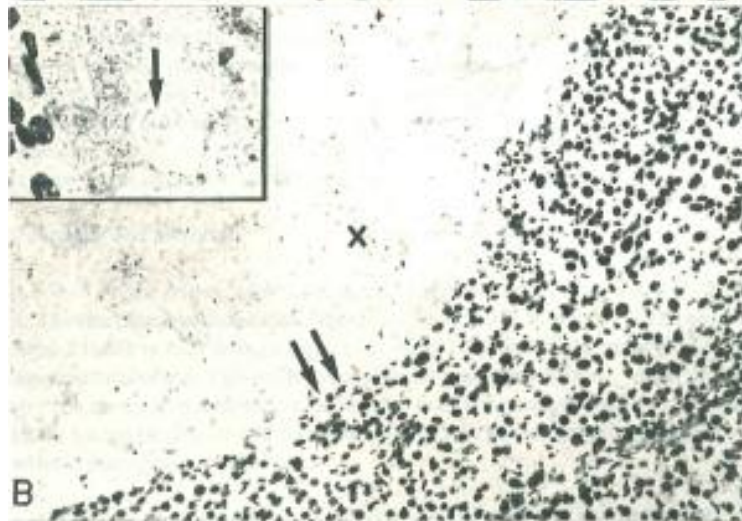
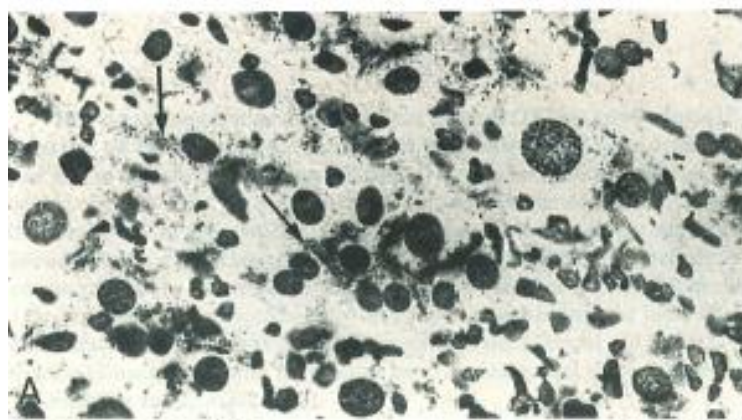
- a. For the degree of the histochemical activity of nucleases, see footnote of table XXIII.
- b. This very high activity concerns surface epithelium
- c. This relatively high activity is limited to the parietal, and zymogen cells, whereas surface epithelium is practically negative.

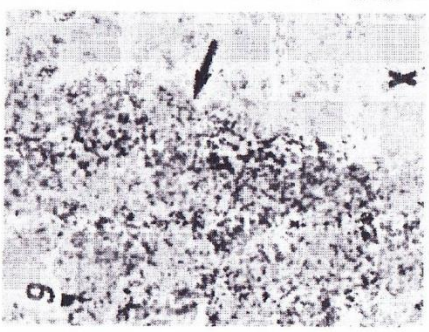
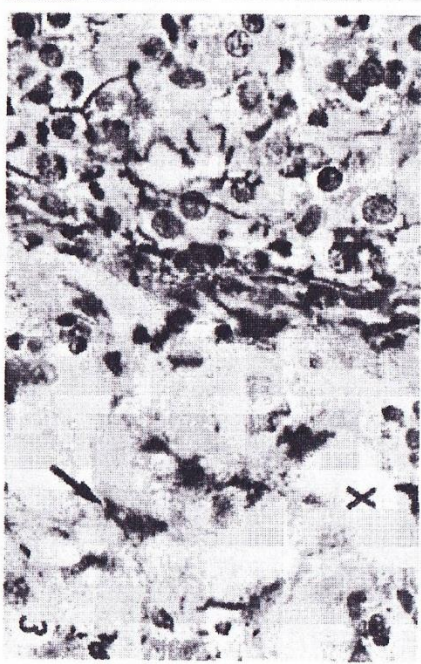
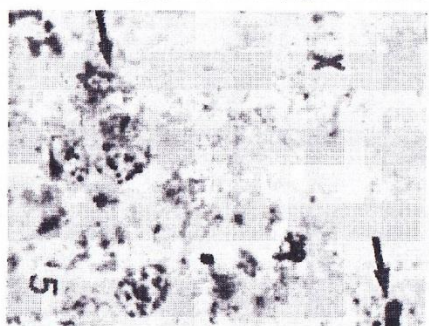
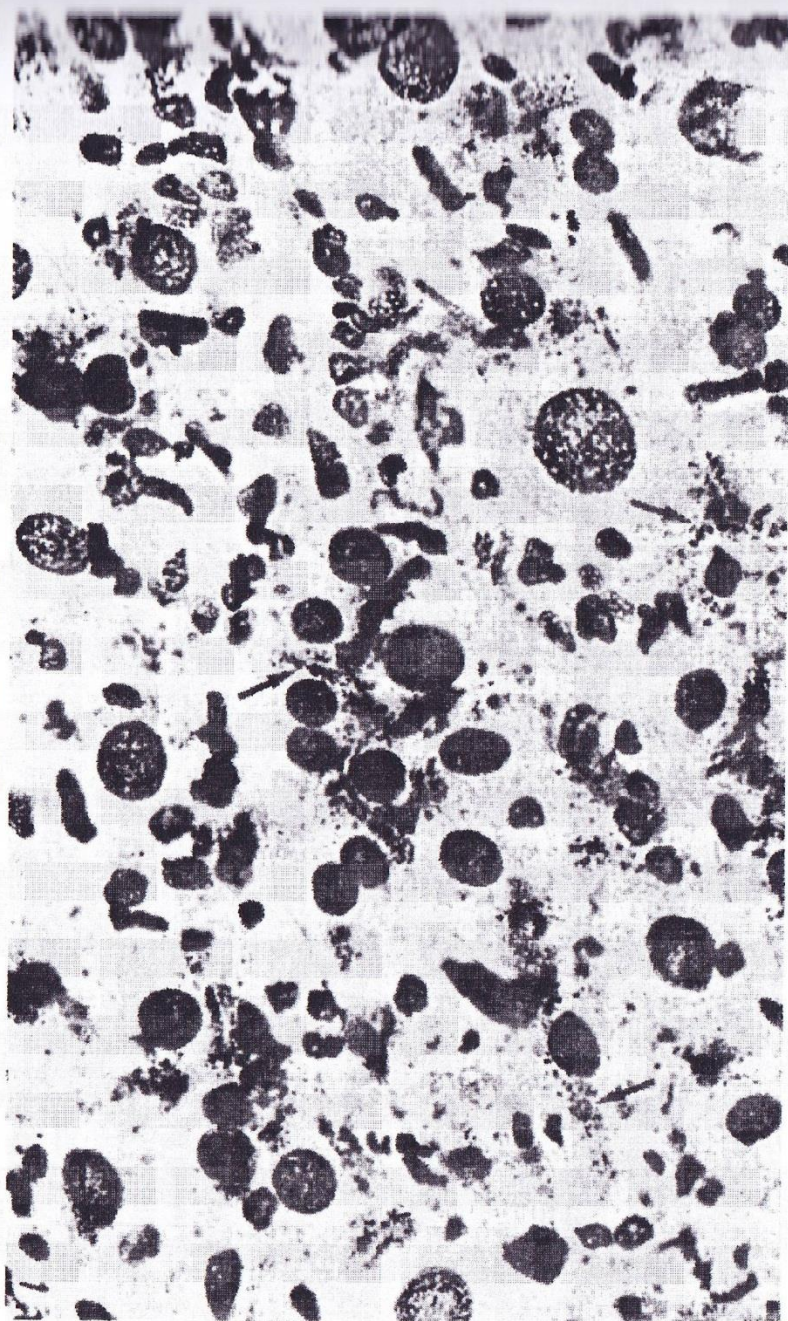
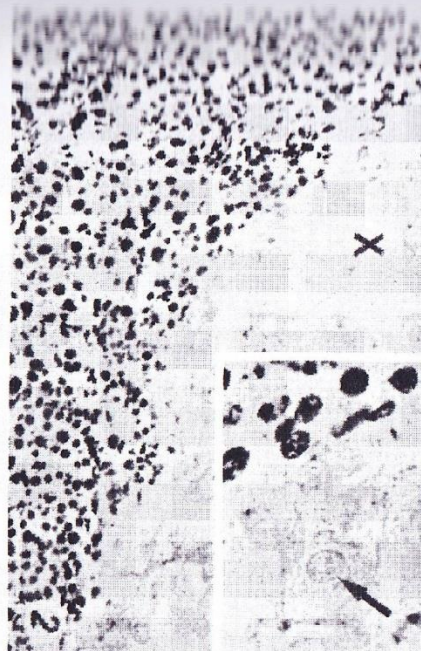
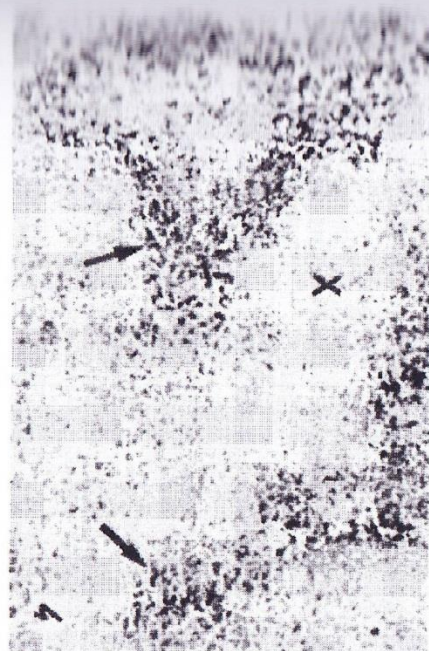
3.DNA alteration does not induce a perturbation in a cell as long as the cell maintains its protective mechanisms, i.e. its nucleases' activity. Its absence may be congenital such as in XERODERMA PIGMENTOSUM or induced by environmental effect, i.e. tobacco or bile salts, etc.



To follow up, one induced cancer in the rat liver by adding one drop of nitrosomorpholine in drinking water during 10 days. This product provokes a methylation of guanine of the DNA and, following Phenobarbital treatment, as a promoter, during one month. As a result, all rats died from liver cancer after 3-4 month period. This lapse of time corresponds more or less at the same evolution of 30-40 years in human being. Those rats were also systematically studied with histopathology and one found that after 38 days, nuclear and cytoplasmic dysmorphism and, after 125 days post treatment, a total disappearance of nuclease activities and a blossoming of tumours (**Dia 12**).



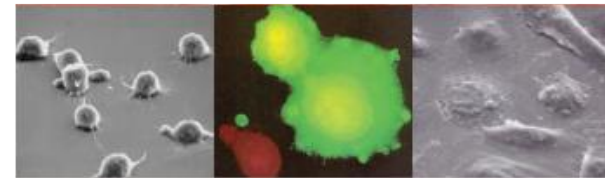




An example of a metastatic oesophagus tumour in the brain with its acid and alkaline nuclease detection is illustrated (**Dia 15**). One can see that the tumour tissue is negative for acid and alkaline DNases as well as for the RNases while the stroma showed a strong

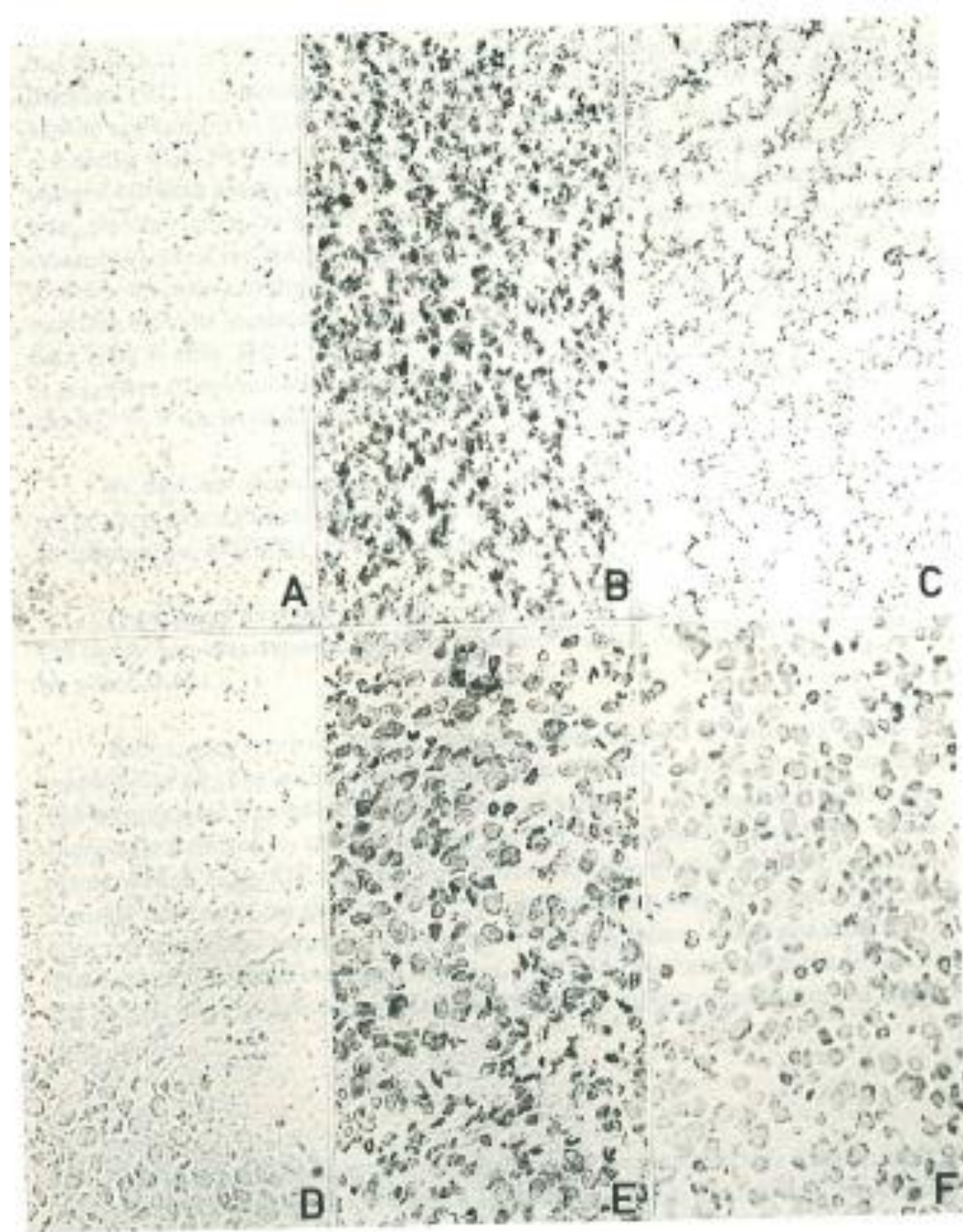


4. In neoplastic tissues, nucleases are present but inhibited. The autolysis first attacks the inhibitory component. This component could act through oxidation.



In Dia 16, if one studies the hepatoma, it is negative for the acid and alkaline DNases. After one hour of incubation and autolysis these nucleases are reactivated and these activities disappear after 4 hours.

All seems to indicate that the inhibition can be obtained by an oxidation at the level of the sulfhydryl groups, for example. This action may change the stereochemistry of the enzymes.



We then develop together a methodology with tissue slices that one can immerse in test tubes. This would allow to study other reactive compounds. Henryk Taper called this way of procedure 'modo Fort'. We studied several reduction products at diverse concentrations and we found that ASCORBIC ACID is reactive at the level of the cytoplasm while MENADIONE was at the level of the nucleus. Henryk then suggested to combine both compounds and to study their best ratio as CK3.

5. As a result of transplantation in mice of the ' TAPER LIVER TUMOR (TLT) ' mice died after 10 days.

However, injection of a small quantity of CK3 (30/1.6mg/day/10days in 5ml saline water – in control only saline water) as soon as the ascite is formed, allows to double the survival and some mice survived indefinitely.

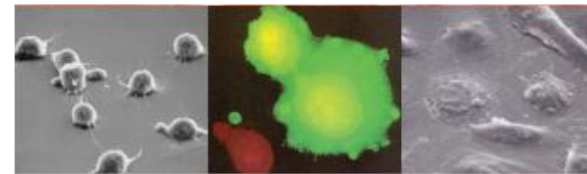
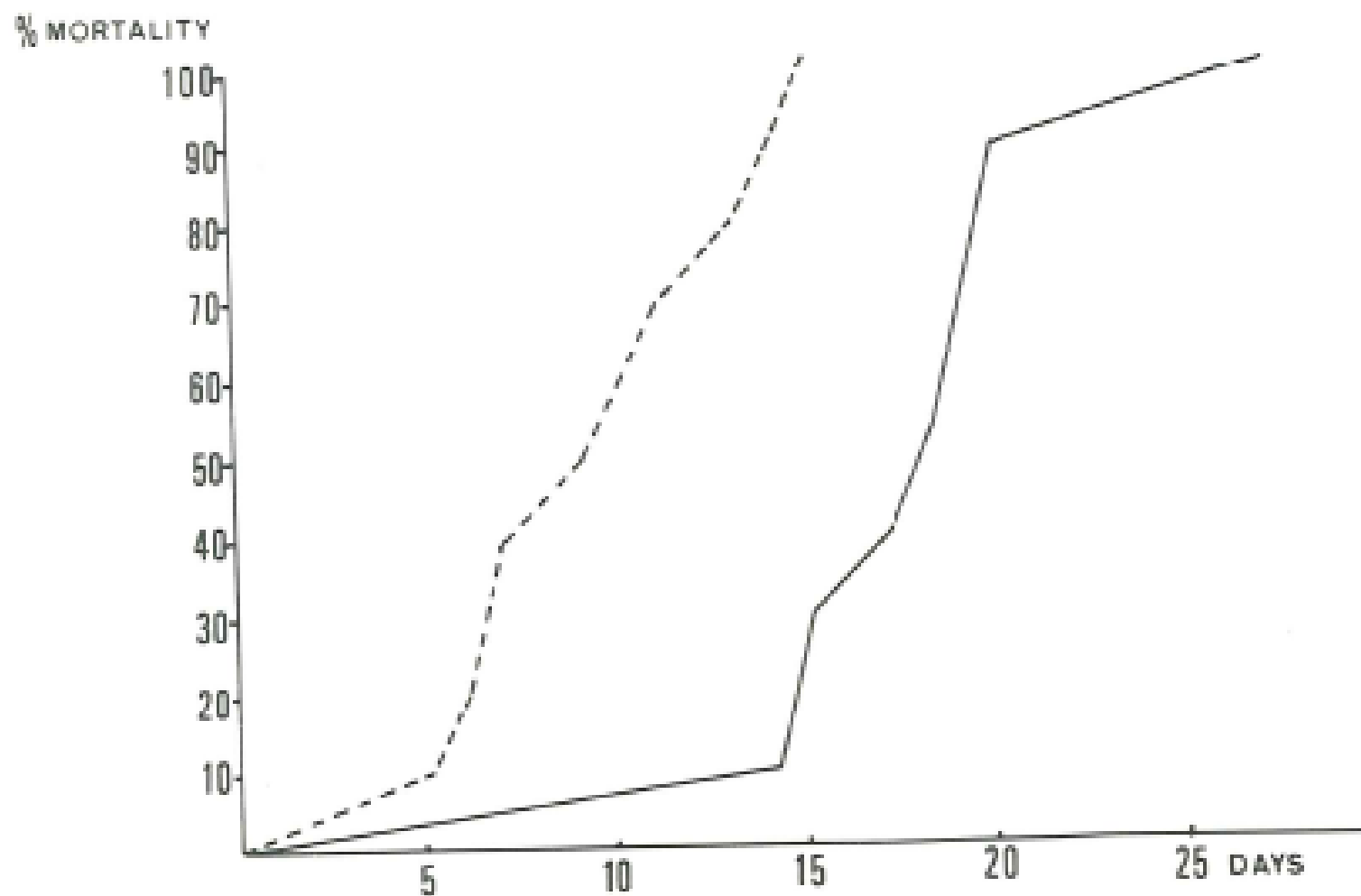
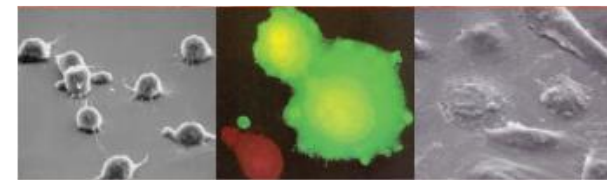


TABLE XV SURVIVAL RATE OF MICE WITH INTRAPERITONEALLY TRANSPLANTED ASCITES HEPATOMA.

---- = untreated (control) ; — = intraperitoneally treated with vitamin C and menadione sodium bisulfite



- **References:** compiled by J. Gilloteaux out of PubMed for symposium references
- Fort L, Taper HS. 1969. [Histocytochemical activity of alkaline and acid nucleases, compared to the incidence of carcinomas in the digestive system of the rat](#) [French] Pathol Eur. 4(1):42-57.
- Fort L, Taper HS, Brucher JM. 1969. [Nucleases activity in different segments of the human digestive tube compared to the incidence of carcinomas \(histochemical study\)](#). Histochemie. 20 :150-8
- Taper HS, Brucher JM, Fort L. 1971. [Activity of alkaline and acid nucleases in tumors of the human central nervous system. Histochemical study.](#) Cancer. 1971 28(2): 482-90.
- Taper HS, Fort L, Brucher JM. 1971. [Histochemical activity of alkaline and acid nucleases in the rat liver parenchyma during N-nitrosomorpholine carcinogenesis.](#) Cancer Res.31(6):913-916.
- Fort L, Taper HS, Brucher JM. 1974. [Gastric carcinogenesis in rat induced by methylnitrosourea \(MNU\). Morphology, and histochemistry of nucleases.](#) Z Krebsforsch Klin Onkol Cancer Res Clin Oncol. 81(1):51-62.
- Fort L, Taper HS, Brucher JM. 1977. [Morphological alterations and focal deficiency of the histochemical activity of acid and alkaline nucleases in rat liver after chronic administration of phenobarbital.](#) Beitr Pathol. 161(4):363-375.
- Taper HS, Lans M, de Gerlache J, Fort L, Roberfroid M.1983: [Morphological alterations and DNase deficiency in phenobarbital promotion of N-nitrosomorpholine initiated rat hepatocarcinogenesis.](#) Carcinogenesis. 4(2):231-234.



In Conclusion:

All of our experiments never contradicted our concept to carcinogenesis and our hope was to have made some strides against it (Dia 17).

Le Nobel de médecine à trois Américains



The Nobel Assembly at Karolinska Institutet has awarded

The 2009 Nobel Prize in Physiology or Medicine

jointly to

Elizabeth H Blackburn, Carol W Greider

and Jack W Szostak

for the discovery of

**"how chromosomes are protected by
telomeres and the enzyme telomerase"**

More information and publications on
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