phosphorylation; furthermore, it is known that proteins like miosine and actomiosine, which are of essential importance to the miofibrilles, cannot be built up in the absence of vitamin E, the vitamin which also prevents the oxidation of stored lipids and liposoluble vitamins[2].

A possible correlation was considered between the biochemical modifications induced by vitamin E and the morphological changes inside the cell, which could be best detected by using electron microscope methods.

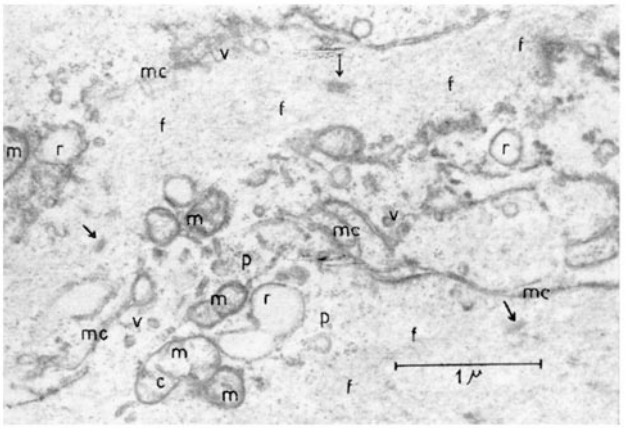

Fig. 1. Miometrial preparation from castrated rabbit, × 20000.

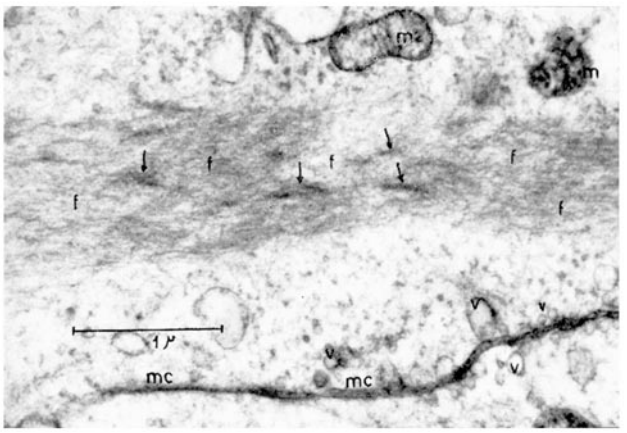

Fig. 2. Miometral preparation from castrated rabbits treated with vitamin E. × 20000. f miofilaments, m mitochondria, c mitochondrial crests, mc cell membrane, r endoplasmic reticulum, v pinocytosis vacuoles, p ribosomes.

The experiments were carried out on ovariectomized rabbits. A group of operated animals were injected subcutaneously daily with vitamin E, at the dose of 60 mg for 20 days. Samples of the uterus wall were fixed in osmic acid according to the method of PALADE[3] and included in Vestopal[4]; sections obtained with a Porter-Blum microtome were examined under a Philips EM 100 A electron microscope.

Animals treated with vitamin E show morphological differences from ovariectomized controls both in macroscopic and microscopic examinations of the uterus: the muscular layer is enlarged, and this to a considerable extent.

Samples from the two groups of animals also differ markedly at electron microscope level. The behaviour of the miofibrilles is the most impressive feature: in smooth cells of the castrated control animals, these structures are reduced to a fine fibrillary network with no specified distribution and no microfilaments visible singly. This aspect is probably due to the fact that the muscular proteins are not completely developed and oriented. Dark bodies are always present and they can be interpreted either as non-contractile proteins, or as fusion points of several fibrilles (Figure 1).

In vitamin E treated animals, uterine smooth cells are well-developed: they run along the whole length of the cell, are well-organized in compact bundles and miofilaments show a well-defined resolution. Dark bodies are increased in number and size (Figure 2).

In conclusion: Vitamin E exerts an elective stimulus on the synthesis and organization of muscular contractile proteins of the uterine wall.

These morphological data are in very good agreement with the biochemical evidence already available of such action of vitamin E.

Riassunto. Il trattamento con vitamina E di conigli ovariectomizzati determina una ipertrofia dell'utero alla quale corrisponde a livello ultracellulare uno sviluppo particolarmente evidente delle proteine contrattili muscolari.

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Istituto di Farmacologia e di Terapia, Milano (Italy), May 29, 1962.

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Behavior of Human Carcinoma Reminiscent of Virus[1]

Histological evidence for transformation of normal liver cells located in the neighborhood of carcinomatous neoplasms, be they of hepatocellular origin (hepatoma) or metastatic to the liver has been reported in a total of 58 cases by ELIAS[2-4] and by ELIAS, SHERRICK and BOULDIN[5]. The epithelial cells of renal tubules behaved identically in the one case of a metastasis to the kidney which was studied. The metastatic growths (22 cases) included in the above mentioned observations had originated at the following primary sites: oesophagus epithelium, stomach, colon, lung, prostate, breast, endometrium, pancreas, and vulva epithelium.

Histological sections of these tumors including large portions of the host organ were submitted to three stains: hematoxylin and eosin; WILDER's uranium and

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silver impregnation[6]; and fluorescence after acridine orange stain, a method originated by SCHUEMMELFEDER[7] and modified for paraffin sections of formol fixed material by ABRAMS and ELIAS[8].

The cancer infested organ can be divided into four zones which are particularly well defined when the tumor has an expansive appearance, i.e., when it is almost spherical in shape. The tumor is designated as zone 4 (zona maligna). It is surrounded by a narrow zone of compressed liver (or kidney) cells which, in spite of compression, still form a typical hepatic muralium of basically normal structure or typical renal tubules. This typical and in essence structurally normal appearing parenchyma is continuous with the tumor; and transformation of its cells into cancer cells have been clearly demonstrated. This parenchyma which adjoins the tumor directly is designated as zone 3 (zona determinata). External to that zone follows a broad area of parenchyma structurally perfectly normal. This is zone 2 (zona affecta). The remainder of the organ is occupied by what must be considered at this time to be truly normal parenchyma. We call it zone 1 (zona sana).

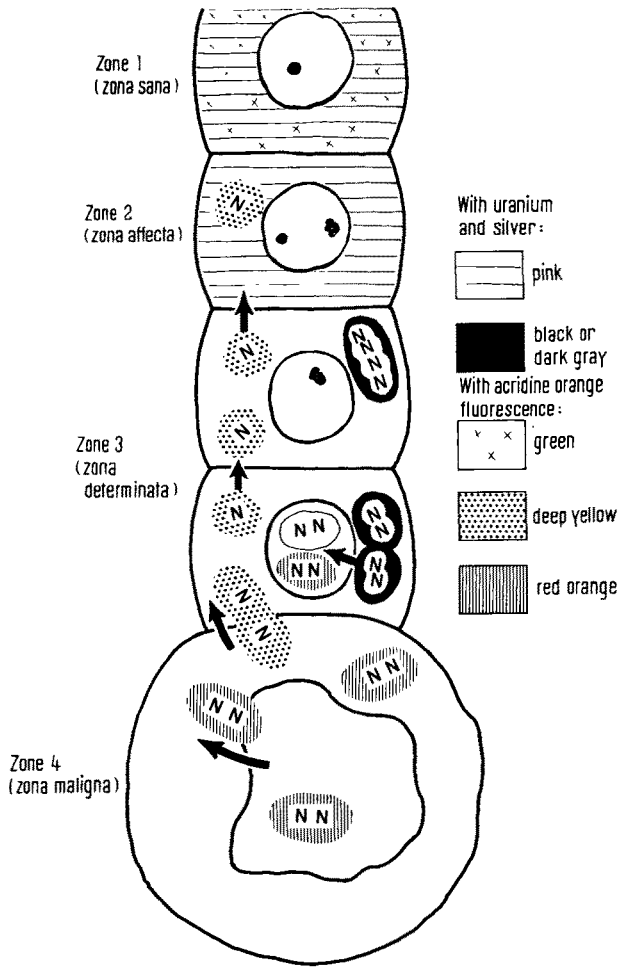
While after acridine orange stain the nuclei of all zones fluoresce red-orange, while their nuclear membranes and chromatin particles become black with the uranium and silver method, and while they stain ultramarine blue with hematoxylin, there are marked differences in the staining qualities of the *cytoplasm* among the four zones. These differences in staining reaction must be ascribed to cytochemical alterations in zones 2–4. They will presently be described (and this description refers to the parenchymal cytoplasm only).

Zones 1–3 are characterized by uniformly acidophil cytoplasm, in other words, they stain red with eosin, while zone 4 (the tumor itself as well as a few isolated tumor cells incorporated into the structurally normal parenchyma) is very slightly basophil. Zone 1 (zona sana) fluoresces green after acridine orange stain. Zones 2 and 3 fluoresce deep yellow after acridine orange stain, while zone 4 fluoresces red-orange.

After impregnation with uranium and silver according to WILDER, zones 1 and 2 stain pink, zone 3 stains black or very dark gray. Often the blackness is very finely granular or diffuse throughout this relatively narrow zone; but occasionally, the outer layer of zone 3 contains coarse, black granules with a fine granularity or diffuse argyrophilia nearer to the tumor. The tumor itself becomes occasionally light gray, but is more frequently quite argyrophobe, i.e., its cytoplasm remains perfectly clear.

An interpretation of these phenomena has become possible because of recent advances in virology (FOUCHET and OSTOYA[9]).

These differences in staining reaction suggest an interpretation such as is schematically presented in the figure.



Let us assume that cancer is characterized by a change in the genetic endowment of the nucleus without, for the moment, asking what has caused this genetic change. The cancer cell differs from the normal in that it possesses a specific pair of genes or DNA molecules, symbolized in the Figure as a double N shaped figure. These NN molecules replicate themselves and many of them are extruded into the cytoplasm of the cancer cell imparting it the red-orange fluorescence. They also neutralize the originally alkaline cytoplasm, thus destroying its acidophilia.

Let us further suggest that the NN molecules fall apart into smaller units, presented in the Figure as simple N shaped symbols. These N molecules are small enough to pass through cell membranes and are therefore able to diffuse for a considerable distance into the parenchyma of the host organ. Thus many of them permeate the entire zones 3 and 2. It is these N molecules that produce deep yellow fluorescence.

N molecules in zone 2 are rather recent arrivals, while at a smaller distance from the tumor, i.e., in zone 3, they are more numerous and many of them have been present in this zone for a longer period of time. This prolonged sojourn has given many N molecules an opportunity to aggregate, but in an arrangement different from the original one. This aggregated condition is symbolically expressed by N shaped figures which sit on top of each other. These aggregates of N molecules provide condensation centers for the precipitation of silver and thus produce argyrophilia.

Subsequently, we may speculate, the N aggregates fall apart again and their parts penetrate into a normal nucleus. It is within the normal nucleus that the N molecules re-arrange themselves to reconstitute NN molecules of the original structure. With this last step they have imparted 'genetic information' to a formerly normal

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nucleus, inducing it to behave malignantly as well as forcing it to replicate new NN molecules.

If this hypothesis should be verified by biochemical methods, human carcinoma would assume the aspect of a virus disease, although the origin of that virus remains, for the time being, unknown.

Zusammenfassung. Veränderungen der Reaktion gegenüber Silber und Acridin-Orange-Fluorescenz im Parenchym, um eine metastatische Geschwulst, gestatten die Annahme eines Virus-ähnlichen Mechanismus bei der Übertragung carcinogener Eigenschaften von Zelle zu Zelle.

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Spores of Microorganisms Penicillin-Induced Destruction of Sporulating Cells of *Bacillus cereus*

Duguid's[1] primary hypothesis, that penicillin specifically interfers with the formation of rigid cell wall component, was verified by Park[2-4], who found accumulation of uridine nucleotides in penicillin-treated Gram-positive bacteria. These substances are bound with hexosamine, acetyl-muramic acid and specific peptides[5,6]. The authors presume that penicillin inhibits the transfer of these hexosamin-peptidic formations from nucleotide to the cell wall. Penicillin also interfers with the cell wall synthesis in Gram-negative bacteria, where incorporation of the compounds like diaminopimelic acid into nucleotide precursors of the cell wall is affected[7,8]. Interference of penicillin with the cell wall synthesis causes the formation of osmotically labile forms partially or completely deprived of the rigid layer of the cell wall, i.e. 'protoplasts'[9,10] or 'spheroplasts'[11]. Penicillin seems to be a relatively specific inhibitor of the synthesis of 'basal structure' of the cell wall in both types of microorganisms[12].

Cell wall synthesis is always linked with the cell division, so that penicillin can inhibit mainly the growing culture. Földes and Merétey[13] have described lysis (or protoplasts formation in hypertonic medium) of the growing cells of penicillinase-producing strain of *Bacillus cereus* by penicillin-treatment, while after the end of growth penicillin has no effect on the cells.

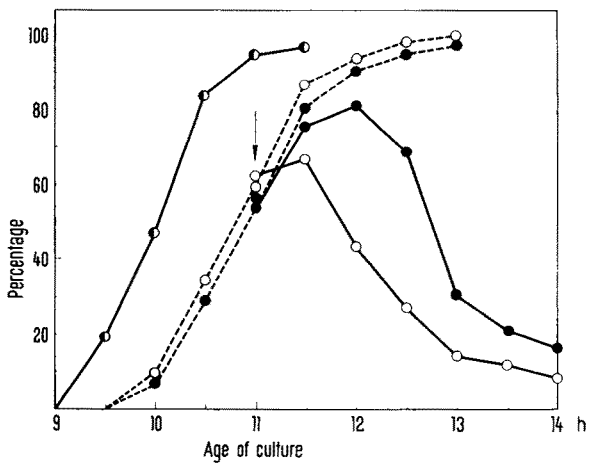

Fig. 1. Release of typical spore constituents after addition of penicillin to sporulating cells of *Bacillus cereus*. Abscisse; age of culture (h); ordinate: percentage. All the values are expressed in percentage, values of control culture at 14 h of cultivation being taken as 100%. (They were for number of spores 96%, for ^{45}calcium content 7300 cpm/mg of dry weight of sporangia and for dipicolinic acid content 30 µg/mg dry weight.) Dashed lines: control culture; full lines: penicillin-treated culture (addition of penicillin designed by arrow); o——o spore formation; ●——● dipicolinic acid content; o---o ^{45}calcium content.

Sporulation of bacilli is a special process, by which a new form of cell-existence spore is formed in the mother cell after the end of growth, equipped with complex protective mechanisms including extremely rigid envelopes. This interesting and curious analogy of the cell division attracts attention with regard to the possible effect of penicillin.

In examining the effect of this antibiotic on sporogenesis, the strain of *Bacillus cereus* (NCIB 8122) was used. It was cultivated in liquid medium containing bactopeptone (0.3%), glucose (0.1%), phosphates and trace elements[14] at 30°C, the culture being aerated by shaking. Three criteria were used for testing the effect of penicillin on sporulating cells:

(1) Synthesis of the component typical for spores of bacilli-dipicolinic acid, tested by the method of Janssen, Lund, and Anderson[15].

(2) Incorporation of ^{45}calcium ($^{45}CaCl_2$ was added to the culture after the end of growth, 0.166 µC/ml, final concentration 2.10^{-4} *M*). Methods of washing of cells by 0,001 *N* hydrochloric acid and determination of radioactivity were the same as in recent work[16].

(3) Morphology of developing spores and their final appearance after release from sporangia.

Penicillin was added in high amounts (1.000 units/ml) to the culture, in which 95% of cells had contained more or less refractive prespores (Figure 1), and during the thermostabilizing process characterized by ^{45}calcium accumulation in cells and dipicolinic acid synthesis (Figure 2) started to pass through. After addition of penicillin, dipicolinic acid continued to synthetise; but,

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