Department of Oncopathology of the State Institute of Oncology (Head : Prof. B. KELLNER)

HISTOLOGICAL CHANGES, AND THEIR CHRONOLOGICAL SEQUENCE INDUCED IN TRANSPLANTED TUMOURS BY NITROGEN MUSTARD, MUSTARD GAS, AND BCM[1,6-BIS (β-CHLOROAETHYLAMINO)-1,6-DESOXY-D-MANNITE-DICHLORHYDRATE]

B. KELLNER

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During earlier investigations the histological and cytological effects induced by some agents capable of influencing cell division have been studied in organs of the rat (colchicine : KELLNER and MATKÓ, 29 ; podophyllin : HOLCZINGER and KELLNER, 24 ; nitrogen mustard : SUGÁR and KELLNER, 52 ; urethane : HARASZTI and KELLNER, 21). These studies have led to a number of observations, some referring to points that are fairly well known, others dealing with phenomena that have so far not received the attention they merit. Since the findings to be discussed in the present paper rest upon the observations made on the organs and are, so to say, their continuation, it is considered desirable briefly to restate the more salient points.

1. The morphological alterations induced by the drugs studied are the most marked in organs with a high rate of mitosis (duodenum, blood, bone marrow, lymphatic system, testes).

2. Definite changes arise in all the cells of the organism, including equally the cells in interphase in the above-enumerated tissues, and the cells in organs where mitosis is uncommon (liver, kidneys, myocardium, etc.). Nuclear lesions, above all, evidence the action of the drugs : the nuclei enlarge or decrease in size (MATKÓ, HOLCZINGER and KERESZTURI, 44), they become loose in structure ; the nucleoli, too, grow larger (GÁTI, 15). But, at the same time, the cytoplasm also displays changes : it turns lighter, the mucus production intensifies, etc.

3. After some time (6, 12, 24 hours), a typical nuclear injury presents itself in many tissues : not infrequently the entire nucleus shrinks to a pyknotic mass, but more often minute globules of chromatin are formed, which for a great part are carried off by the lymph flow, while many are engulfed on the spot by histiocytes.

4. The most significant feature, we hold, is the great regularity with which the morphological changes run their course. In mitotic nuclei the *initial changes* manifest themselves as early as in 15 to 30 minutes after treatment, many injured mitoses are seen, and normal mitoses grow gradually less in number. The *maximum morphological effect* of the treatment develops between the 4th and the 48th hour, and is readily recognised by diffuse nuclear disintegration and changes in

the cells and the nuclei. In dependence on the drug, the organ, and the dose, it lasts broadly speaking for from 6—12 to 96—120 hours. It is followed by *restitution*, with the appearance of an ever increasing number of apparently normal mitoses, an occasional amitosis or giant cell still present, with the cells swelling, and the basophilia of the cytoplasm intensifying.

The investigations carried out on organs were really experiments preliminary to our proposed studies on tumours. The drugs applied being known to give rise to grave morphological changes in tumours as well, it was deemed necessary to establish the existing similarities and discrepancies in the histological picture of, and the course run by, the alterations appearing in the tissues of the organs and the tumours, respectively. Since in tumour chemotherapy the greatest practical significance attaches to the mustard-gas derivatives, and because the compounds which nitrogen mustard forms with sugars had already been studied by us for some time (VARGHA, 54 ; KELLNER, NÉMETH and SELLEI, 30), we gave our first attention to this particular group.

Material and methods

Much labour had to be spent before a more or less suitable method could be evolved by which to follow up the morphological changes in tumours. The one we ultimately used is described here somewhat at length, because despite its awkwardness and the many sources of error inherent in it, it lends itself fairly well to the detection of the nature of effects exerted by the drugs, and to an estimation of their efficacy.

The tumours intended for histological studies were transplanted to inbred animals of uniform weight (rats of 120 to 150 g, and mice of 18 to 25 g in weight). Involved in the investigations were Guérin's carcinoma of the rat, M-1 rat sarcoma, Crocker's S-180 sarcoma of the mouse, and Ehrlich's mouse cancer. In respect of each type of tumour we determined the time of growth at which it is best worked up histologically. This was done by preparing sections of the largest diameter and examining them for the minimum necrosis and necrobiosis, respectively. 7 to 10 days old tumours, in mice the size of a filbert and in rats that of a small walnut, were found to be the most serviceable. At the beginning of the treatment the tumours were all of the same size, they grew uniformly and rapidly. Of nitrogen mustard (methyl-bis-[β-chloroethyl] amine hydrochloride, HN_2), the animals were given 1, 2, and 3 mg per kg body weight intraperitoneally and intravenously ; of mustard gas, 2 mg/kg were administered percutaneously ; while of BCM, 100 mg/kg were injected intravenously. The animals were sacrified 15, 30 minutes, 1, 2, 3, 4, 6, 12, 18 hours, and 1, 2, 3, 4, 5, 6, not infrequently 8 to 10 days, after treatment. It followed from the nature of these experiments that it was not always possible to abide by time limits fixed in advance. At the beginning of each experiment and at the time the last treated animal was killed, an untreated control tumour was also worked up. The organs were fixed in neutral formalin. The sections were cut along the largest diameter of the tumour. Staining was performed with haematoxylin eosin, Giemsa stain, the Feulgen reaction, Mallory's tripledye, picrofuchsin, and Gomori's silver impregnation method.

In addition to the tumours, the organs were worked up, which show typical cytological reactions (duodenum, lymph nodes, spleen, occasionally thymus, bone marrow, testes, etc) : this was done with a view to providing a valid basis for comparing the morphological changes taking place in the tumours and organs, respectively.

Not content with merely registering the chronological sequence of the morphological changes, we aimed at quantitative results. This aim was primarily approached by mitotic counts. The number of normal mitoses, injured mitoses, and multinuclear giant cells found in 500 or 1000 cells in florid portions of the tumour, was ascertained each time. Particularly great technical difficulties were encountered in estimating cellular destruction due to the action of the drugs. Planimetry had to be resorted to, as for the time being there seemed to be no other (biological or chemical) method available. But microscopic planimetry is well known to harbour

very potential sources of error; besides, for our case it proved to be more than usually questionable. So, it was known from the outset that our method is very cumbersome and suitable only for assaying very wide differences. However, the changes, too, are of a very wide scope and so the method furnishes telling information in spite of the difficulties attaching to it. For a better understanding of the technique of this count it need to be mentioned that within the tumour we distinguish four different areas (Fig. 12), namely, the florid (F), the dissociated (D), the pycnotic (P) area, and the necrotic (N) portions of the tumour. The changes of course show an exceedingly great variety of intermediate forms. Using sections cut in the largest cross section of the tumour, and applying a magnification of 40×15, the proportions are then estimated in which each of these four areas is represented in each of 200 or 400 consecutive quadratic visual fields along the two longest and most characteristic diameters. A whole field was taken to be 100% ; half a field, 50% ; a quarter of a field, 25% ; and only discrepancies of not less than 25% were included in the estimate.

Of the tumours studied Guérin's tumour was found to be the most serviceable for our purposes. It is of uniform growth, and its cells are fairly constant in shape and size. The necroses in it are relatively extensive, but always form continuous and rather centrally located areas, and since the tumour consists of contiguous florid portions, an assay is greatly facilitated. Atypical divisions, amitoses, and giant cells are few in number (Table I).

Table I

Guérin's carcinoma in the rat. Untreated control. Tumours 8 to 14 days after transplantation the size of from a filbert to a small walnut. Figures in the same row refer to the identical tumour

F per cent	Mitoses per cent	Amitoses and multinuclear cells per cent
53	0,30	0,8
46	0,42	0,20
53	0,50	0,22
45	0,56	0,0
44	0,54	0,0
59	0,62	0,10

Results

Like in the organs, in the tumours, too, the *initial changes* consist in mitotic injuries, which are characterized primarily by the negative feature that the dividing cells are unaltered in shape and size. The chromosomes are clumped together disorderly, arranged skeinlike conforming to the equatorial plate ; not infrequently, they aggregate in spindle-shaped groups or form a homogenized mass with the chromosome ends sticking out of it like spinules. Less frequent than these essentially pyknotic nuclear changes are injuries which are rather of a lytic character, such as chromosome fragmentation, scatter of the chromosomes over the cytoplasm, their crumbling into powder, etc.; at anaphase, bridge formation or a comblike connection between the receding equatorial plates is not uncommon (Fig. 1). In our view, a greater significance than to these minute karyological changes attaches to the fact that there is a marked reduction in the number of dividing cells, and within this number in that of

normal mitoses. It is not always possible to distinguish injured from normal mitoses, wherefore it is preferred to present the data on the total number of mitoses in the diagrams (Figs. 2 and 3); this total number decreases very considerably between the 2nd and 6th hour.

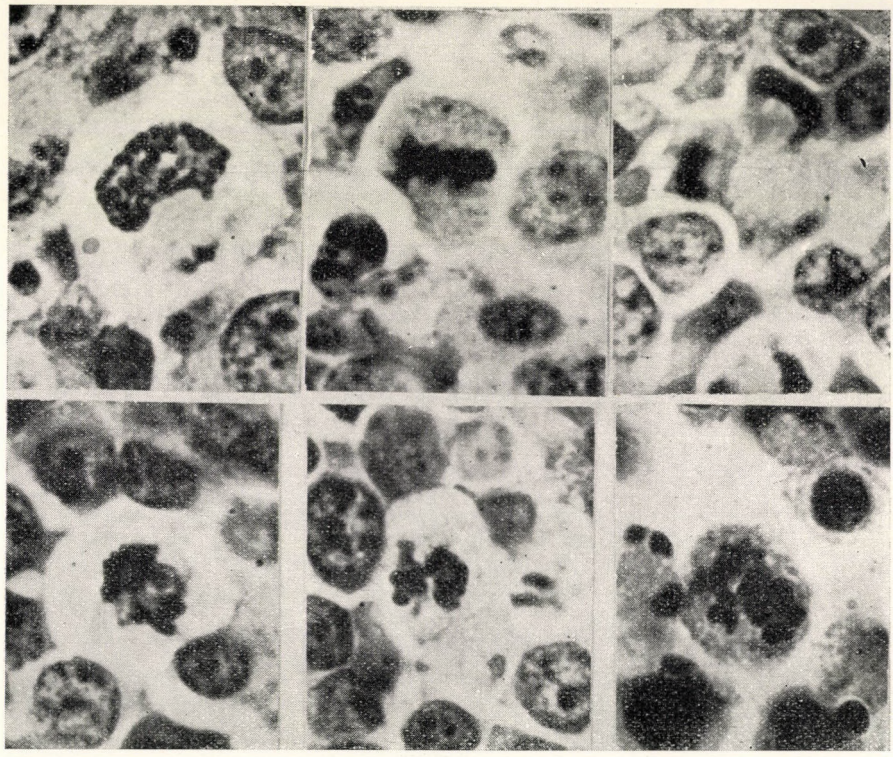

Fig. 1. Incipient mitotic injuries induced by nitrogen mustard (between the 3rd and 6th hour). — 2 mg/kg. — Guérin rat tumour (×1900)

1. Abnormal prophase; chromatin clumped into cluts.
2. Abnormal metaphase; conforming to the equitorial plates, chromosomes congested in skeinlike disposition.
3. Abnormal telophase with irregular aggregation of chromosomes.
4. Abnormal anaphase; chromosomes stick together; comblike connection between the equatorial plates; at the bottom, chromosomes begin to clump into cluts or small spherical mass.
5—6. Practically the whole of the chromatin clumped into spherical mass; structure reminiscent of chromosomes only at places

The *period of maximum effect* is more variegated, and signified by morphological changes which are much more typical. As early as in 12 to 24 hours after the administration of the drug there appear *deformed mitoses (cacomitoses)* (Fig. 4). The cells grow to 3 or 4 times their original size; the chromosomes are individually and distinctly recognisable, and more than normal in number

(aneuploidism). Sometimes they are found in a coil in the centre of the cell, at other times scattered irregulary over the light-coloured cytoplasm. Often they display a stellate arrangement, giving rise thereby to forms transitional towards multipolar divisions. Occasionally, they settle underneath the cell membrane parallel to the surface. The individual chromosomes likewise undergo changes in shape. Frequently they are large and like solid rods, or the shape of a comma ; fragmentation is not uncommon, and less so is their shrinkage to globules. Cells of this type are devoid of any structure ; no centrioles, no mitotic

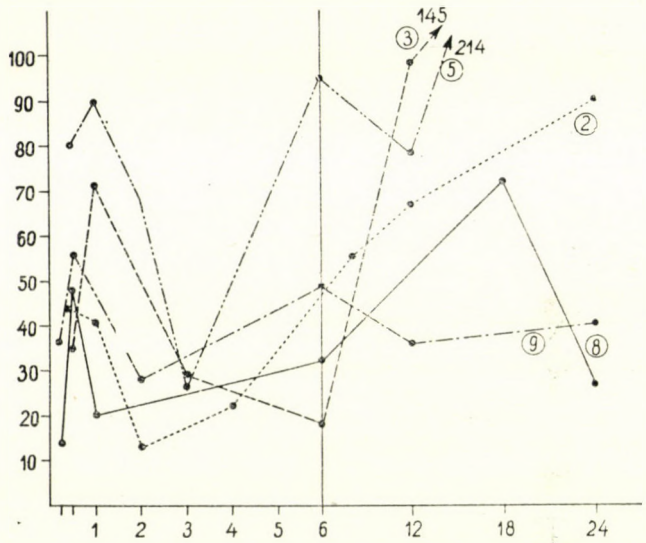

Fig. 2. Changes in the mitotic count in the first 24 hours upon the effect of HN_2. (Normal+abnormal mitoses in 0,1 per cent.)

2. — — — — — — — M-1 rat sarcoma ; 2 mg/kg, i. v.
3. — — — — — — Crocker S 180 mouse sarcoma ; 2 mg/kg, i. v.
5. —..—..—..— Guérin/carcinoma ; 2 mg/kg, i. v.
8. ———————— Guérin carcinoma ; 1 mg/kg, i. p.
9. —.—.—.—.— Guérin carcinoma ; 3 mg/kg, i. p.

Abscissa : time in hours.
Ordinate : total number of mitoses in 0,1 per cent

spindles, can be observed ; the cytoplasm is faint, occasionally frothy. It is not uncommon for the chromosomes to settle in a circle in the cell, giving the impression that fresh nuclei have begun to form. The incidence of anomalous forms is great ; it may attain 10 to 15% of the total number of cells.

Even more striking is another form of mitotic disturbance, wich leads to the formation of *multinuclear tumour cells* (Fig. 5). In the untreated Guérin tumour the proportion of amitotic and multinuclear cells is about 1 to 2%, not exceeding 2 or 3% in Crocker's S—180 and in M—1 sarcoma. But when the mustard effect develops, they keep on growing in number parallel with the

appearance of the cacomitoses. Between the 24th and the 96th hour every second or third tumour cell, at least, is bi- or multinuclear (Fig. 6). Thereafter, their number begins to decrease rather rapidly until, in a week or two, the initial level is approached once more. On repeated dosage of the drug, some are encountered even many weeks later. The histological pictures point to a great many modes of their formation. Sometimes scattered chromosome parts appear to arrange themselves to a nucleus in the protoplasm, at other times forms reminiscent of multipolar division lead to the formation of giant cells. Fairly frequent

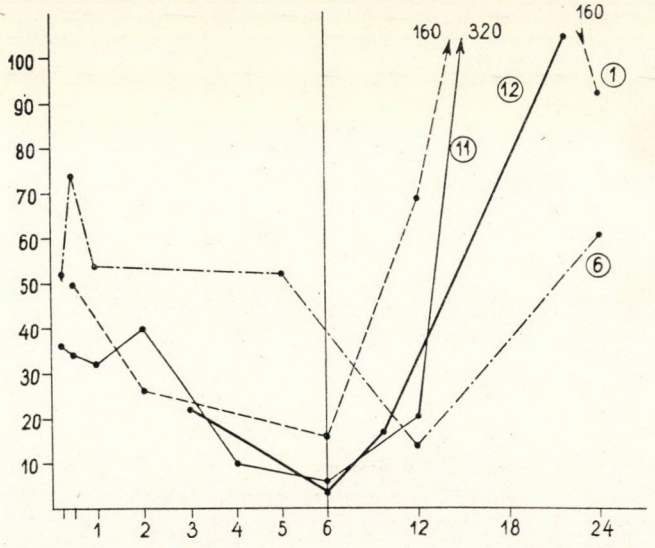

Fig. 3. Changes in mitoses in the first 24 hours on effect of mustard gas and BCM, respectively.
12. ——————— mustard gas ; 0,2 mg/kg, percutaneously. Guérin rat carcinoma
1. — — — — — BCM ; 100 mg/kg, i. v. Guérin rat carcinoma.
6. —.—.—.—.— BCM ; 100 mg/kg, i. v. Crocker S 180 mouse sarcoma.
11. ——————— BCM ; 100 mg/kg, i. v. Guérin rat carcinoma

is endoamitosis, when cleavage of the nucleus, or its disintegration into many nuclei, occurs within the nuclear membrane. Not uncommon is the dumbbell form typical of amitosis. In other instances, nuclear portions penetrate through the nuclear membrane into the cytoplasm. However, these histological pictures equally permit the a sumption that the chromatin globules arising in consequence of nuclear disintegration are engulfed by the tumour cells, and that it is the latter that somehow have some part to play in the formation of new nuclei. It is not exceptional to find amitosis and mitosis side by side in the same cell.

Nuclear or cellular destruction and disintegration start as early as 30 minutes or one hour after the administration of the mustard derivatives, and is the most typical characteristic of the onset of the period of maximum effect.

The nucleus is often seen to disintegrate into pyknotic chromatin granules, which at first are found in the central region of the cell taking the place of the nucleus, and later are scattered over the entire cytoplasm, to be encountered ultimately

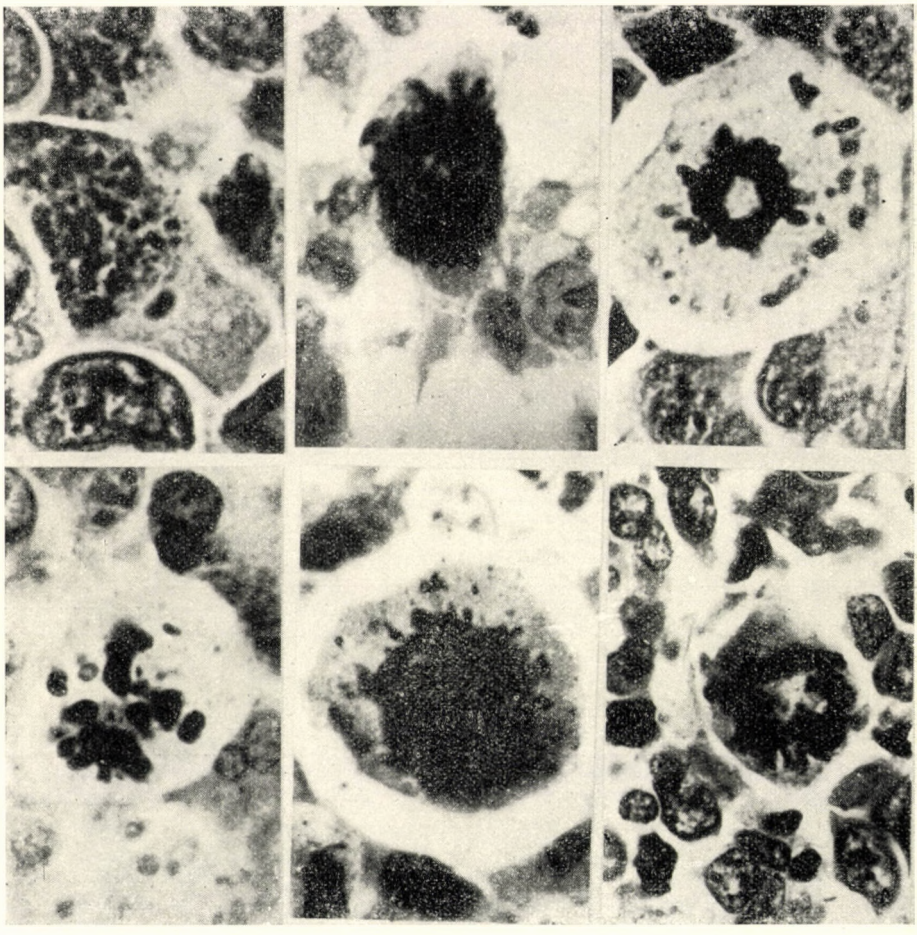

Fig. 4. Mitotic abnormalities (cacomitoses) upon the action of HN_2 during the period of maximum effect (between 24th and 72nd hour); 2 mg/kg, i. v. (×1900)

1. Cell increased to multiple of original size, with chromatin and chromosome disposition corresponding to metaphase, fairly irregular, aggregated and clumped.

2. Nucleus consisting of clumped chromosomes, with some chromosome ends sticking out of it.

3. Chromosomes disposed in a circle conforming to equatorial plate; great number of chromosomes scattered in cytoplasm, particularly beneath the cell membrane and parallel with it.

4. Chromatin clumped, not infrequently into spherical mass, and scattered chromosomes.

5—6. Centrally disposed mass of chromosomes and scattered chromosomes in cytoplasm

outside the cell, naked, or surrounded by a narrow and indistinct protoplasmic strip. Frequently, the nucleus shrinks to a pyknotic spherical mass of some size,

which, when it is set free from the cytoplasm, turns into a so-called naked nucleus. The minute granules encircled by a narrow strip resemble cellular structures hardly 1 or 2 μ in size (Fig. 12). Like in normal tissue, their fate cannot be followed ; in part, they are carried off by the flow of lymph ; in part, histiocytic

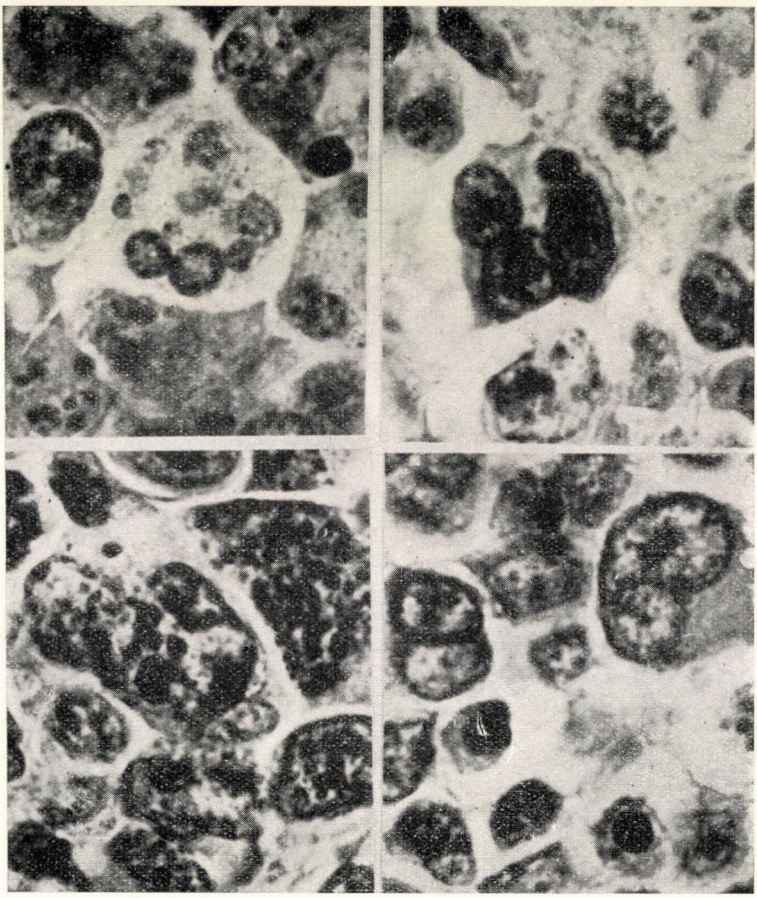

Fig. 5. Multinuclear tumour cells forming upon the action of HN_2 in the period of maximum effect and the restitutive period, respectively (between 48th and 144th hour). ($\times$ 1900)

1. Cell showing nuclear structure corresponding to prophase and many times the normal size, in which the chromatin is in several places so disposed as if several new nuclei were forming. In the cytoplasm a single chromatin globule.

2. Several nuclei of varying size and grade of maturation forming in the cytoplasm.

3. Abnormal amitosis in one of the cells. Above : binuclear cell ; right : chromatin globules and pyknotic masses of chromatin.

4. Multinuclear giant tumour cells, with nuclei of varying maturedness and a single spherical mass of chromatin

elements of the stroma ingest them. Apparently, many of them are taken up by the tumour cells. Cellular destruction being a constant phenomenon in

tumours, the cell-damaging effect exerted by the mustard derivatives can only manifest itself in a difference in degree, which the method described is unable to display. Typical of the effect of mustard derivatives is an increase in the number of visual fields showing disintegrating cells in the florid portions, scattered singly or in small groups. This type of alteration develops to an extent where after the treatment there are practically no intact florid portions in the tumour, and nothing but pyknotic disintegration is seen among the tumour cells

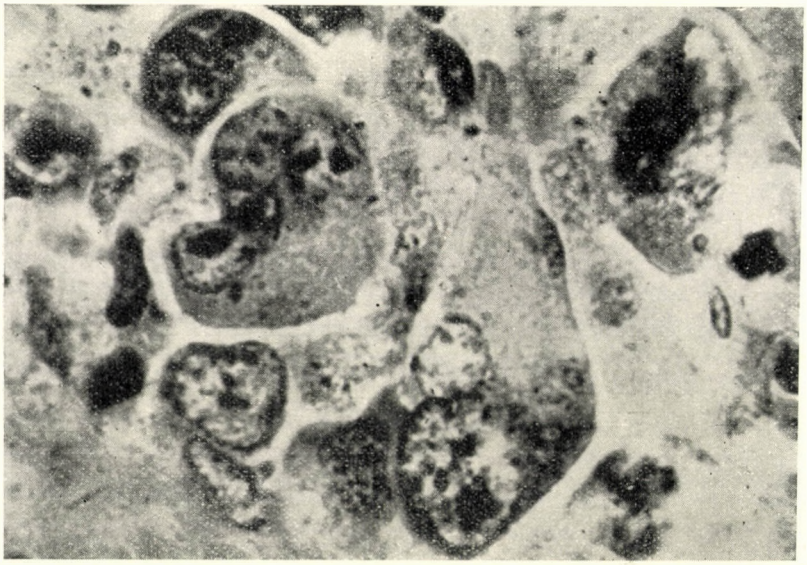

Fig. 6. Giant-cell formation in Guérin rat carcinoma 24 hours after the administration of BCM ; 100 mg/kg, i. v. (×1900)

retained. (In the planimetric count this finds its expression in a great frequency of visual fields in which the proportion of F is 75 and that of P 25%, or that of F and P 50% alike.) (Figs. 7, 8, and 9.)

In organs the above mentioned changes of cells in interphase may be regarded as corresponding to *the enlargement and shrinkage, respectively, of the cells in tumours.* While most of the cells and their nuclei grow larger and lighter, there are many tumour cells which shrink, their nuclei shrinking with them. The nucleoli sometimes enlarge, sometimes assume a stellate, more often an irregular, shape ; not infrequently, they are of loose texture.

The *restitutive process* is less difficult to follow in tumours than organs. From the florid portions first there begin to disappear the dying forms, and with them the deformed mitoses. It is considerably later that the number of amitoses and multinuclear cells decreases, and that of apparently normal mitotic forms gradually increases.

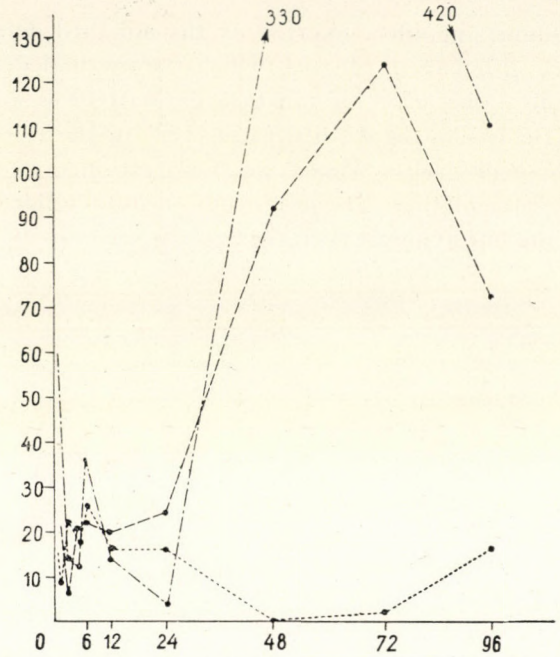

Fig. 7. Changes in total number of mitotic forms on effect of HN_2 (3 mg/kg ; Guérin rat cc)
— — — — — — — Normal mitoses
— — — — — — — Abnormal mitoses
—.—.—.—.—.— Amitoses and giant cells
Normal mitotic count dropping early below 2%; no normal mitosis between 48th and 72nd hour ; number of mitotic abnormalities attaining a very high value at the same time (between 48th and 96th hour). From 24th hour onwards every second or third tumour cell multinuclear

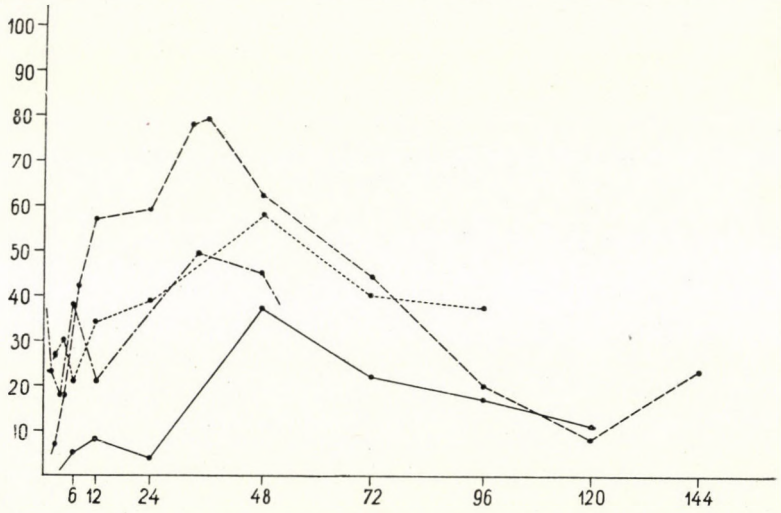

Fig. 8. Intensification of pyknosis on effect of HN_2
9. — — — — — — — Guérin rat carcinoma ; 3 mg/kg, i. p.
5. —.—.—.—.— Guérin rat carcinoma ; 2 mg/kg, i. v.
2. — — — — — M-1 rat sarcoma ; 2 mg/kg, i. v.
3. ——————— Crocker S 180 ; 2 mg/kg, i. v.

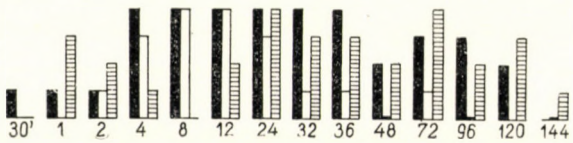

Fig. 9. Course run by morphological changes in the duodenum consequent upon administration of HN^2, 2 mg/kg, i. v.

Three basic changes are illustrated for each point of time.

Black column : reduction in number of mitoses and mitotic injuries.

White column : degree of nuclear disintegration.

Shaded column : changes of glandular epithelium (intensification of mucus production, growing and shrinking of cells and their desquamation, appearance of multinuclear forms) Height of columns indicates degree of change, +, +, ++ and +++. The alterations induced by HN_2 in tumour and duodenum are seen to run largely the same course

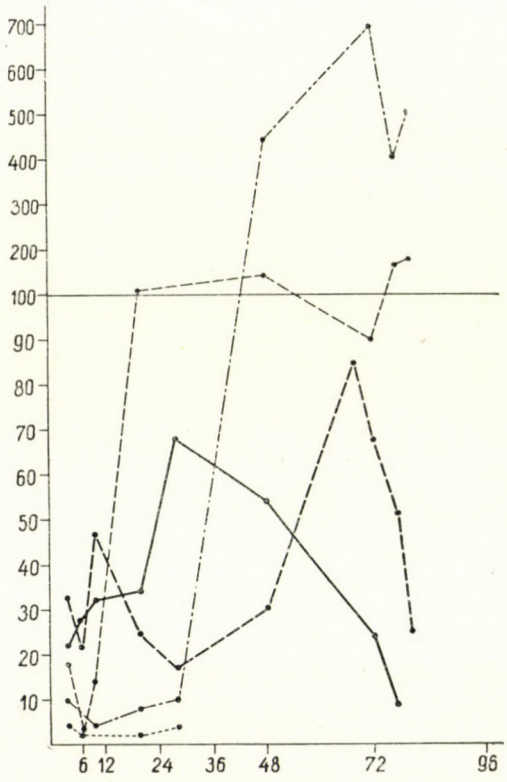

Fig. 10. Course run by morphological changes induced by mustard gas ; 0,2 mg/kg, percutaneously ; Guérin rat carcinoma.

———————— Proportion of necrotic areas in percentage of the whole tumour
— — — — — — Proportion of pyknotic areas in percentage of the whole tumour
— — — — — — — Normal mitoses, %
— — — — — — Abnormal mitoses, %
—.—.—.—.—.— Multinuclear cells, %

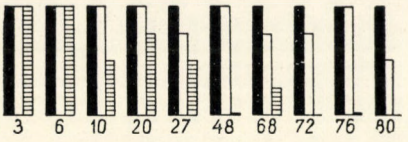

Fig. 11. Course run by morphological changes induced in the duodenum by mustard gas. Dosage and denotations as above. Severe nuclear changes presenting themselves during the entire experimental period; marked nuclear disintegration and cellular destruction

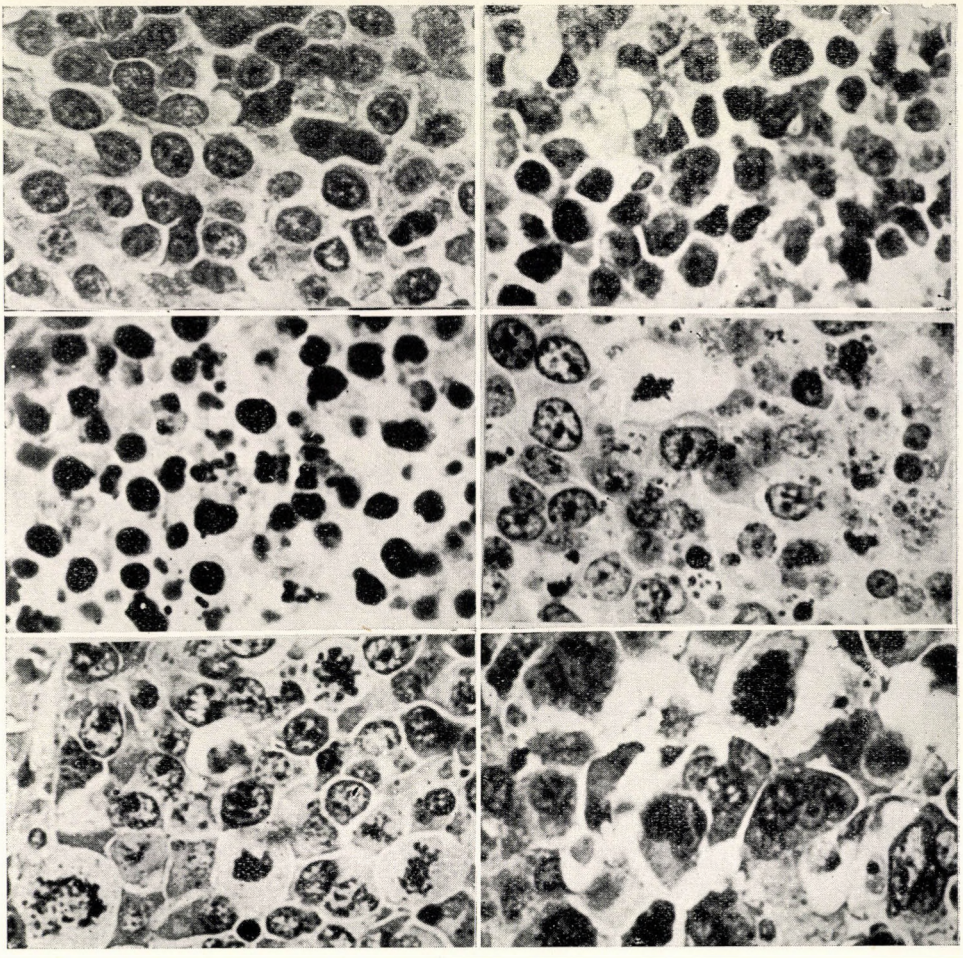

Fig. 12. Histological changes arising on the effect of BCM.

1. Florid portion of untreated Guérin tumour.
2. Dissociation. Structure of tumour cells retained, bnt connection between them lost; 12 hours.
3. Pyknosis. Nuclei of tumour cells shrunken and structureless; 12 hours.
4. Nuclear disintegration visible. Minute chromatin globules forming, frequently surrounded by protoplasmic brim. A very few mitotic abnormalities seen. Growth and shrinkage of nuclei conspicuous. 33 hours.
5. Great number of mitotic abnormalities (cacomitoses). 33 hours.
6. Tumour consists entirely of amitoses and multinuclear tumour cells. Cells few and far between with reticulate connective tissue. 120 hours (H. E.; ×1600)

What has been said above refers primarily to our findings for nitrogen mustard. The other two compounds studied in detail (mustard gas and BCM) were found to give rise to morphological alterations essentially similar to those

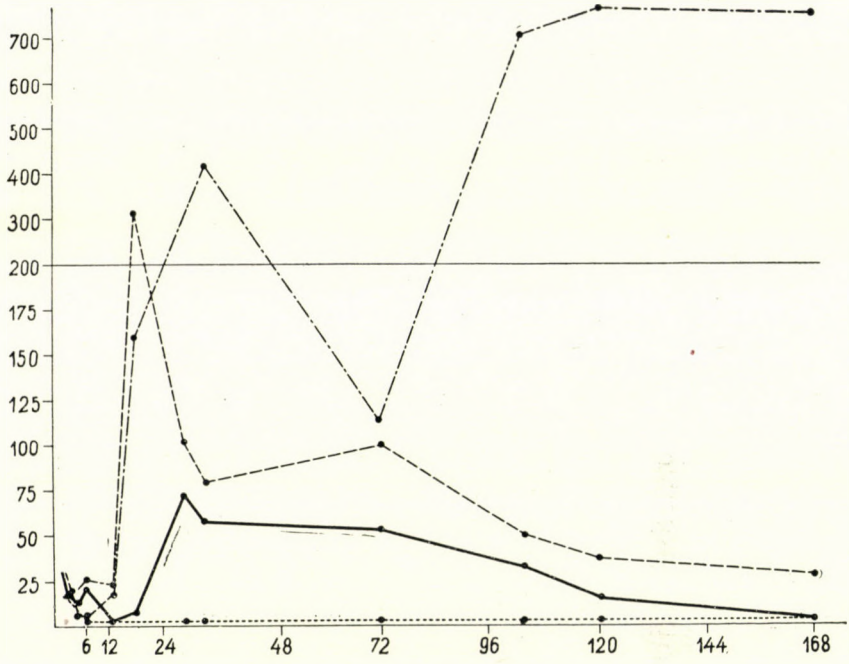

Fig. 13. Course run by morphological changes induced by BCM ; 100 mg/kg, i. v.; Guérin rat cc.
——————— Percentage distribution over the whole tumour of visual fields filled entirely by florid portions containing pyknotic cells and cell groups
— — — — — — — Normal mitoses, %
— — — — — — — Abnormal mitoses, %
—.—.—.—.—.— Giant cells, %

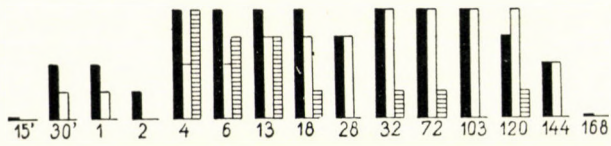

Fig. 14. Course run by morphological changes induced by BCM in the duodenum of the rat ; 100 mg/kg, i. v.

achieved with nitrogen mustard. Whatever differences there were, may find their explanation in differences of the doses and the absorptive conditions. Yet the changes elicited by *mustard gas* were graver, and they ran a somewhat quicker course (Figs. 10 and 11). Late alterations could not be observed, the animals having died in 3 or 4 days.

BCM (1,6-bis [β-chloroaethylamino]-1,6-desoxy-D-mannite-dichlorhydrate) is less toxic than HN_2; it is stable, and therefore easy to dose exactly (KELLNER and NÉMETH, 31). The initial changes develop as rapidly, and the maximum effect is of the same duration, as in the case of nitrogen mustard, but the restitutive period is longer (Figs. 12, 13, and 14). These facts account for it that it was BCM which could be administered most easily and most satisfactorily over a long period. BCM was found to inhibit tumour growth up to more than 90% ; the tumours of treated animals weighed less than 10% of the control tumours, while the loss of body weight was not more than 10%. The treated tumours consisted almost in their entirety of a necrotic, respectively pyknotic mass, with a seemingly increased amount of connective tissue within it. This we consider relative, for we have failed to detect active proliferation of the connective-tissue elements. Retained tumour cells were seen only on the periphery, usually among bundles of connective tissue, disposed like strings of beads. Occasionally, perivascular tumour cells were encountered. Treated tumours consisted to more than nine tenths of such necrotic or necrobiotic portions. The remaining tumour cells were mostly bi- or multinuclear, but not infrequently all kinds of deformed mitoses could be seen. On discontinuing treatment the tumours began to grow, and after some time the picture turned into one of an untreated tumour.

It has been mentioned that apart from Guérin's tumour other transplanted tumours have been treated with the agents under discussion. Experiments have been carried out on rat sarcomas induced with benzopyrene, and spontaneous mammary cancer cases not yet regardable as completed. The morphological changes observed in these tumours, and the course run by them, agreed in essence in every respect with those observed in the Guérin tumour.

Discussion

The morphological and cytological changes induced by mustard gas and mustard derivatives, particularly nitrogen mustard, have been studied in different kinds of tumours and viewed from various aspects (KARNOFSKY, BURCHENAL, ORMSBEE, CORNMAN and RHOADS, 26 ; BLOCK, SPURR, JACOBSON and SMITH, 4 ; BOYLAND, CLEGG, KOLLER, RHODES and WARWICK, 5 ; GAENSLER, MCKAY, WARE and LYNC, 14 ; GRAEF, KARNOFSKY, JAGER, KRICHESKY and SMITH, 19 ; SPITZ, 49 ; GOLDIN, GOLDBERG, ORTEGA, FUGMANN, FAIMAN and SCHOENBACH, 18 ; LANDING, GOLDIN, NOE, GOLDBERG and SHAPIRO, 35 ; KARNOFSKY, 27 ; SHAPIRO, GOLDIN, LANDING, BERGNER, FAIMAN and GOLDBERG, 47). Studies have been made primarily of those nuclear changes which are regarded by us as initial changes (GILMAN and PHILIPS, 16 ; KOLLER, 34 ; GOLDACRE, LOVELESS and ROSS, 17 ; BIESELE, PHILIPS, THIERSCH, BUR-

CHENAL, BUCKLEY and STOCK, 3 ; HEILMEYER, 22 ; LOVELESS and ROSS; 42 ; KOLLER and CASARINI, 33 ; KOLLER, 32 ; LETTRÉ, 40 ; MARQUARDT, 43; ROSS, 46). These cannot be regarded as characteristic ; in untreated tumours a considerable proportion (3 to 5%) of the dividing forms is known to show fairly similar alterations (KOLLER, 32 ; VOUTILAINEN, 55). In judging the initial effect, it is in our opinion of greater significance that the mitotic forms which can be regarded as normal disappear almost completely, and that the injured mitoses, too, are few in number. This makes it understandable why between the 2nd and 6th hour the proportion of the total number of dividing forms instead of being 3 to 7%, is only 1 to 2%, or even less. This early substantial drop in the number of mitoses has been observed also by other authors, not only in tumours (SHAPIRO, GOLDIN, LANDING, BERGNER, FAIMAN and GOLDBERG, 47) but in ascites as well (HEILMEYER, 23). More typical is the morphology of deformed mitoses (cacomitoses). Again, we consider their marked numerical increase during the period of maximum effect to be more characteristic and of greater significance than the changes that take place in their structure. That the number of divisions still regardable as normal rises to its multiple is in the first line due to the appearance in great numbers of these abnormal mitoses. Various explanations can be invoked : an obvious assumption is that the cells take more time to divide but there is no method available to prove this (WIDNER, STORER, and LUSHBAUGH [57]).It is conceivable that the transformation products of the mustard derivatives forming in the organism are in some way capable of intensifying cell division (S. BRAUN: Karyoplastic Effects, 6) ; on the other hand, owing to their toxic effect they give rise to irregular mitotic forms. Departing from the findings that in the vicinity of the necrobiotic areas dividing forms are encountered in remarkably large numbers (KOLLER, 32), and that the number of mitotic abnormalities rises parallel with the extension of the necrotic, necrobiotic, and regressive processes, respectively, we first of all suggest, and shall try to prove by future investigation, that it is the decomposition products forming on the disintegration of the tumour cells which produce the stimulus to cell division.

More conspicuous even are the amitoses and multinuclear cells. There are several references in the literature to the formation of giant cells on the effect of mustard derivatives, the same as on that of X-ray irradiation. FELL and ALLSOPP [10] observed them to appear in the skin: RINALDINI [45] in explants ; GAENSLER, MCKAY, WARE and LYNC [14] in tumours. According to LETTRÉ and SCHLEICH [38], in ascitic tumours the numerical increase of amitoses coincides in time with the maximum decrease of mitotic forms. Their morphogenesis is exceedingly varied. The forms described are merely examples. Until quite recently they have been linked up with regressive processes, but now the view is spreading that they are to be regarded as a very frequent and very interesting form of cell division. Our suggestion again is that their formation is initiated

through some role played by the decomposition products arising on cellular disintegration. In our view they take a decisive part in the restitutive process, the normalisation of the histological picture. Following up the course supplies evidence that they transform into regular, viable cells. It is by these cell formations that we try to find a consistent explanation for the fact that by means of the drugs studied the tumour cannot be annihilated completely, but after a time starts to grow again from residual portions, principally from those consisting of multinuclear cells. While, chiefly by repeated administration, it is possible to convert the tumour almost in its entirety into a necrotic mass, underneath the capsule, on the edges and along the vessels, there remain a few bizarre-shaped multinuclear cells, which in some unknown way form once more into tumour cells of normal structure, after treatment has been discontinued. Simple morphological means are insufficient to follow up closely, and eventually to verify, the development of these tumour cells and their retransformation into normal cells. Further data concerning this process may in the first line be expected from cinemicrography of explants. The question of toleration from inuredness to these drugs has lately become a point of interest (LAW, 37 ; LETTRÉ, 41) ; it is usually explained by enzymic adaptation. In our opinion this, too, is a question which cannot be studied unless these peculiar cell forms are included in our consideration. Studies of their biological significance are facilitated by the fact that for a fairly long time after the administration of the drugs practically the whole tumour consists exclusively of them. Probably, during this time a change takes place in the tumour's reaction to organic stimuli, to chemicals which find their way into the organism, to the action of irradiations, etc.

The mostly pyknotic, but in part lytic, nuclear disintegration and destruction processes merit attention from more than one point of view. On disintegration, naked nuclei, minute chromatin globules, are formed, occasionally surrounded by a narrow strip of protoplasm. Clumped together, they frequently fashion into vast chromatin pools. While many of them are undoubtedly carried off by the flow of lymph or blood, large masses remain on the spot. They cannot possibly be neutral to adjoining tumour cells, but probably mean an altered chemical environment for them. When carried off to different parts of the organism, they most likely behave there as a foreign substance. Also, there is much to support the idea that on given occasions these cellular fragments and altered cells, respectively, might regenerate into florid cells.

When reporting our experiments on the organs of the rat we have already expressed our view that for the great number of cells perishing in the lymphatic system and the intestinal tract upon the effect of nitrogen mustard, there is no other explanation but that cells in intermitosis are also exposed to the contingency of disintegration. This was emphasized in several addresses delivered at the Second Symposion in Freiburg, principally in respect of the haematoblastoses

and based upon the findings of GRUNDMANN [20] and ALTMANN [1]. Following a single large dose, but particularly repeated administration of the drug, the disintegration of the tumour cells is so wide-spread as to exclude any other conclusion but that of cells in the resting stage being destroyed.

Our above observations concerning the course taken by the morphological changes need to be discussed separately. Changes in blood and bone marrow smears are followed up routinely in laboratory as well as clinical examinations (BLOCK, SPURR, JACOBSON, SMITH, 4 ; LARIONOV, 36). Several authors have succeeded in observing in tissue cultures some of the minute histological alterations. Thus, first of all initial nuclear changes have been followed by FRIEDENWALD, BUSCHKE, SCHOLZ and MOSES [13] on the cornea, by FELL and ALLSOPP [10] on the skin, by WEBBER, CRAIG, FRIEDMAN [56] on the intestinal mucous membrane. By serial observations of the changes in the intestinal mucous membrane DESAIVE and VARETTO-DENOEL [8] have elaborated a method very useful in studying the protective effect of certain substances. Explants have been found to be particularly suitable objects, and several workers have rendered them more appropriate by application of serial photography (FELL and ALLSOPP, 11, 12 ; BASTRUP-MADSEN, 2 ; CORNMAN and ORMSBEE, 7). Like blood smears, ascites tumour has proved a good object by wich to follow cytological changes (TAKIKAWA, HIRAMATSU, *et al.*, 53 ; LETTRÉ and BERGDOLT, 39 ; YOSHIDA, 58). Some authors have endeavoured to find means by which to turn these qualitative changes into quantitative values. Of outstanding interest are in this respect the studies by KINDRED [28] concerned with the lymphatic system. The literature also contains references to attempts at following up the changes taking place in solid tumours (SHEAR, 48 ; SHAPIRO, GOLDIN, *et al.*, 47 ; ZHDANOV, 59 ; STOCK, 50, 51 ; DUNN, 9).

In the absence of systematic studies with methods like ours this form of successive cell destruction and cell formation had been unknown. Our investigations revealed that upon the effect of the mustard derivatives there soon arises a substantial change in the structure of the tumour merely to regress in a few days. Of course, the process cannot be followed unless during administration of the drug systematic histological examinations are made at short intervals. Practically every point of time furnishes its own typical morphological picture. This fact alone should suffice to make plain why in attempts at ascertaining the efficacy of these drugs or the lack of it, histological tests have not enjoyed as much appreciation as they would seem to merit. Investigations now in progress show that the dose applied conclusively influences the morphological picture. The histological effect changes and is dependent on whether the identical agent is given once or on several occasions, as also on the intervals at which it is administered (HOLCZINGER, 25). LETTRÉ and BERGDOLT [39] have demonstrated that in ascites tumour cells the same dose of N-methylcolchicamide gives rise to quite different cytological changes if administered at once and in fractions.

Systematic investigation into the dynamics of these morphological changes might also furnish some footing on which to place a more rational application of the combinative treatments which now have the vogue. Following the metabolic changes and their comparison with simultaneously obtained histological pictures represent another line of research which, by the testimony of our first tentative experiments, is promising good results.

Summary

The histological changes induced by mustard derivatives follow characteristic courses. It has been possible to disclose them by working up transplanted tumours 15, 30, 60 minutes, 3, 6, 12, 24 hours, and daily, respectively, after the administration of the drugs. For the purposes of the examinations Guérin's carcinoma of the rat has proved the most suitable.

At the beginning of the drug action the mitoses are injured and the total number of dividing forms decreases (to 1 or 2%). During the period of maximum effect there appear many mitotic abnormalities (cacomitoses); they may attain as much as 10 to 15% of the total number of cells; at the same time, there is an even greater numerical increase in amitoses and multinuclear tumour cells (even up to 50 or 70%). In the florid portions of the tumour extensive pyknotic cellular and nuclear disintegration is observable. During the restitutive process the giant cells remain in the foreground, and apparently give rise to a new cell population. The cacomitoses cede their place to normal mitoses; the chromatin clots and globules, originated from nuclear disintegration, disappear.

A quantitative estimation of these changes has also proved possible. First of all the ratio of mitotic to amitotic forms has been determined and expressed in per cents. In addition, with the aid of microplanimetry, the percentage proportion of pyknotic and necrotic areas in the florid portions has been estimated.

The course run by the changes has been found to vary with the drug and the dose. The initial period generally lasts 6 to 12 hours; the period of maximum effect passes between the 48th and the 96th hour into the period of restitution, the termination of which is very difficult to determine exactly. Vestiges of the changes are demonstrable up to the 5th and 10th day.

The morphological alterations induced by the three drugs studied are essentially similar. The effect of BCM is somewhat protracted. The principal advantage to be gained from following up the dynamics of the morphological changes will show itself in testing the efficacy of new drugs on trial. Knowledge of the course taken by these changes will prove useful in deciding on dosage and combinative therapy. Parallel with the morphological alterations biochemical changes can also be demonstrated.

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ТКАНЕВЫЕ ИЗМЕНЕНИЯ, ВЫЗВАННЫЕ ГОРЧИЧНЫМ АЗОТОМ, ГОРЧИЧНЫМ ГАЗОМ И BCM (1-6-BIS/β-CHLORAETHYLAMINO-1-6 DESOXY-D-MANNIT-DICHLORHYDRAT), И ИХ ТЕЧЕНИЕ ВО ВРЕМЕНИ В ТРАНСПЛАНТИРОВАННЫХ ОПУХОЛЯХ

Б. КЕЛЛЬНЕР

Тканевые изменения, вызванные горчичными производными, проявляют характерное течение. Автору удалось следить за этим изменениям путем обработки трансплантированных опухолей каждые 15 – 30 – 60 минут, или каждые 3 – 6 – 12 – 24 часов или ежедневно после введения исследуемого вещества. Самым подходящим для целей данных исследований оказался рак крыс Герена.

Вначале воздействия повреждаются митозы, число всех делящихся форм уменьшается (до 1 – 2%). При наивысшем воздействии проявляется своеобразный, уродливый митоз (какомитоз, который может достигнуть 10 – 15% всех клеток). Еще больше повышается число амитозов и многоядерных опухолевых клеток (до 50 - 75%). На флоридных участках опухоли наблюдается пикнотический распад клеток или же ядер. Во время восстановительного процесса гигантские клетки еще преобладают, причем из последних образуются, повидимому, новые популяции клеток. Какомитозы сменяются нормальными митозами и хроматиновые глыбы и шарики, образовавшиеся вследствие распада ядер, исчезают.

Автору удалось количественно проследить за этим изменениям. Прежде всего он определил процентное соотношение митотических и амитотических форм на флоридных или же пикнотических и некротических областях.

Течение процесса зависит от исследуемого вещества и от дозы последнего. Начальная фаза длится как правило 6 – 12 часов, максимальное действие переходит около 48 – 96 часов в восстановительную фазу, причем точное определение конца последней фазы весьма трудно. Следы изменений можно выявить еще по истечении 5 – 10 дней.

Исследованные 3 вещества вызывали в сущности одинаковые морфологические изменения. Действие BCM немного протягивается. Динамическое наблюдение морфологических изменений с успехом используется прежде всего при определении, тестировании эффекта исследуемых новых средств. Знание течения хорошо используется при определении дозировки и комбинированного лечения. Параллельно с морфологическими изменениями можно выявить также и биохимические изменения.

DURCH SENFNITROGEN, SENFGAS UND BCM (1-6-BIS[β-CHLORAETHYLAMINO]-DESOXY-D-MANNITDICHLORHYDRAT) VERURSACHTE GEWEBSVERÄNDERUNGEN UND DEREN ZEITLICHER ABLAUF IN TRANSPLANTIERTEN GESCHWÜLSTEN

B. KELLNER

Die durch Senfderivate hervorgerufenen Gewebsveränderungen zeigen einen charakteristischen Ablauf. Die Beobachtung dieser Veränderungen wurde dadurch ermöglicht, dass die transplantierten Geschwülste nach Verabfolgung des zu untersuchenden Stoffes alle 15—30—60 Minuten, 3—6—12—24-Stunden, bzw. täglich bearbeitet wurden. Von den transplantierten Geschwülsten erwiesen sich die Guérinschen Rattenkarzinom am geeignetsten für die obenerwähnten Untersuchungen.

Am Anfang der Einwirkung wurden die Mitosen geschädigt, die Zahl aller Teilungsformen verminderte sich (bis zu 1—2%). Während der maximalen Einwirkung erschienen viele abnormale Mitosen (Kakomitosen), die 10—15% sämtlicher Zellen ausmachen können. In noch grösserem Maße vermehrten sich die Amitosen und die vielkernigen Geschwulstzellen (sogar bis zu 50—75%). In den floriden Teilen der Geschwulst entsteht pyknotischer Zellen- bzw. Kernzerfall. Zur Zeit des restitutiven Prozesses sind die Riesenzellen weiter vorherrschend. Aus letzteren entstehen anscheinend neue Zellenpopulationen. Die Kakomitosen werden von normalen Mitosen abgelöst, und die aus dem Kernzerfall entstandenen Chromatinschollen und -kugeln verschwinden.

Diese Veränderungen konnten auch quantitativ verfolgt werden. Als erstes wurde das prozentuale Verhältnis der mitotischen zu den amitotischen Formen in den floriden Teilen bestimmt. Außerdem wurde mit Mikroplanimetrie das prozentuale Verhältnis der floriden, bzw. pyknotischen und nekrotischen Gebiete schätzungsweise bestimmt.

Der Ablauf hängt vom untersuchten Stoff, und von dessen Dosis ab. Die Anfangsphase dauert im allgemeinen 6—12 Stunden, die maximale Wirkung geht ungefähr um die 48—96. Stunde in die restitutive Phase über, deren Ende nur schwer mit Genauigkeit festgestellt werden kann. Die Spuren der Veränderungen können nach 5—10 Tagen noch nachgewiesen werden.

Die untersuchten drei Stoffe riefen im wesentlichen ähnliche morphologische Veränderungen hervor. Die Wirkung von BCM ist etwas verzögert. Die dynamische Beobachtung der morphologischen Veränderungen kann vor allem bei der Bestimmung, Testierung der Wirkung neuer zu untersuchender Stoffe mit Erfolg angewandt werden. Die Kenntnis des Ablaufs ist bei der Ausarbeitung der Dosierung und der kombinierten Therapie von Bedeutung. Parallel mit den morphologischen Veränderungen können auch biochemische Veränderungen nachgewiesen werden.

Prof. Béla KELLNER, Budapest, II., Ráth Gy. u. 5. Hungary.