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EFFECT OF ACUTE AND CHRONIC NITROGEN MUSTARD TREATMENT ON THE ORGANS OF THE RAT

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The drugs most frequently applied in the treatment of malignant neoplasma are nitrogen mustard and its derivatives. Numerous authors have been concerned with the experimental investigation of the mechanism of their action. The effect was mostly measured by morphologic methods i. e. by examining the changes resulting in the tumour cells and the organs.

V. G. Nemets had been the first [43] to synthesise the drug under the name embichin in 1933. It was then independently produced by *A. Gilman* and *F. S. Phillips* [18]. Its action was examined by *Malyughina* [41], *Zhdanov* [58], and other authors *(Graef, Karnofsky, Jager, Krichesky* [19]), while *B. H. Landing, A. Goldin, H. A. Noe, B. Goldberg,* and *D. M. Shapiro* [37], examined the effect of about 40 derivatives of nitrogen mustard in order to find a correlation between chemical structure and toxicity.

From among the drugs exerting an inhibitory effect on neoplasms, first colchicine, then podophyllin were examined in detail in this Department *(Kellner-Matkó* [32]; *Holczinger—Kellner* [24]). It was endeavoured to observe the morphological changes, to state the time when the effect first set in. We established the duration of the effect in the tissues both after a single dose and on repeated administration. Finally it was important to clear, whether the basic changes produced by nitrogen mustard are similar to, or differing from those produced by colchicine. Colchicine, beside damaging the cells dividing by mitosis, changes the course of development of every cell in the organism. Consequently the problem presented itself, whether or not nitrogen mustards acted in the same manner. Nitrogen mustards usually being administered for a long time, the effect of both a single large dose and several low doses had equally to be observed. After studying the tissue changes produced by nitrogen mustard, colchicine and podophyllin, a further object consisted in working out a basis for a rational combined therapy. According to *B. H. Landing* and his associates [35], more reliable information about the cytotoxic phenomena produced by drugs interfering with tumour growth may be received from normal organs than from the tumour itself. In accordance with that view, first the effect exerted on normal organs was studied, in order to apply the experience gained to tumours.

Material and methods

60 white rats, mostly male, ranging in weight from 100 to 160 g, were employed. The animals were treated by intraperitoneal injections of a 0,08 per cent solution in physiological saline of nitrogen mustard (methyl-bis-β-chloroethylamine hydrochloride, made by Magyar Pharma). The doses administered, the duration of treatment, the interval between the injection and the time elapsed from the last injection until the examination had been performed, were widely varying. 26 animals were used in the acute experiments. 10 rats had been given a single injection of nitrogen mustard in a dose of 200 microgrammes per 100 g body weight and they were killed after a period lasting from 10 minutes to 120 hours. 16 animals were treated with half the above dose and they were killed by bleeding, after 10, 30, 60 minutes, 2, 3, 4, 5, 6, 24, 48, 96 and 120 hours, respectively. The latter dose corresponds to the one employed in chronic treatment. 8 rats were given a daily dose of 100 or 200 microgrammes per 100 g for 2 to 4 days, and then killed 2 to 24 hours following the last injection. In this way an increased acute effect was produced. Finally, 26 rats were exposed to chronic treatment, i. e. the injections were given with doses of 75 to 100 microgrammes per 100 g weight every third day, and subsequently killed between the 7th and the 155th day of treatment, the majority during the 8th or 9th week, always 2 or 3 days after the last injection. 10 animals belonging to the group receiving prolonged treatment were examined 6, 24, 48, 120, 144 hours, 9, 11 and 24 days, respectively, after the last injection. In this way we wanted to observe the destruction and restitution under chronic treatment. According to the results of the acute experiments, it was possible to establish that after every repeated dosage of nitrogen mustard the same destruction and restitution appeared as in the case of a single application.

The injections were followed by a loss of weight amounting from 10 to 25 g, except when lower doses had been given. The animals treated with repeated injections became retarded in growth, their body weight did not increase or even diminished. Histological examinations were performed on the alimentary tract, thymus, lymph nodes, spleen, testicles, epididymides, liver, kidney, lungs, myocardium, pancreas. For fixation, Susa's fluid or a 4 per cent solution of neutral formaldehyde was used. The sections were stained with haematoxylin and eosin, Heidenhain's haematoxylin or Mallory's stain. Papp's silver impregnation and Feulgen's reaction were also used. Best's carmine stain for glycogen was applied to sections of the liver in numerous cases. Organ systems for the examination of which special methods are necessary will not be dealt with in the present paper, their changes will be published at a later time.

Changes observed in organs

Duodenum. At the bottom of the crypts swelling of part of the epithelial cells sets in as early as after 10 minutes. A large mass of mucus is secreted. Cellular damage and destruction could not be established for certain before 1 hour had elapsed. The most conspicuous alteration occurs in mitotic cells although the number of these is greatly reduced. The nuclear substance is arranged in the shape of a V made up of powder-like spherules (Fig. 1 A). Other nuclei consitute a dense irregular meshwork (1 C). In many cells the nuclear substance is situated centrally or excentrically (1 B) and later it becomes dissipated in the cytoplasm (1 C). At the same time, other cells contain larger spheres (1 D).

The picture is rather similar to that brought about in the duodenal mucosa by colchicine. Beside the ones containing powder-like chromatin or larger

chromatoid globules, cells containing 3, 6, 8 or 10 spheres may also be seen. It seems warranted to infer from the pictures that the appearance of spherules represents a new, second phase of cell damage (Fig. 1 D). If the globules are

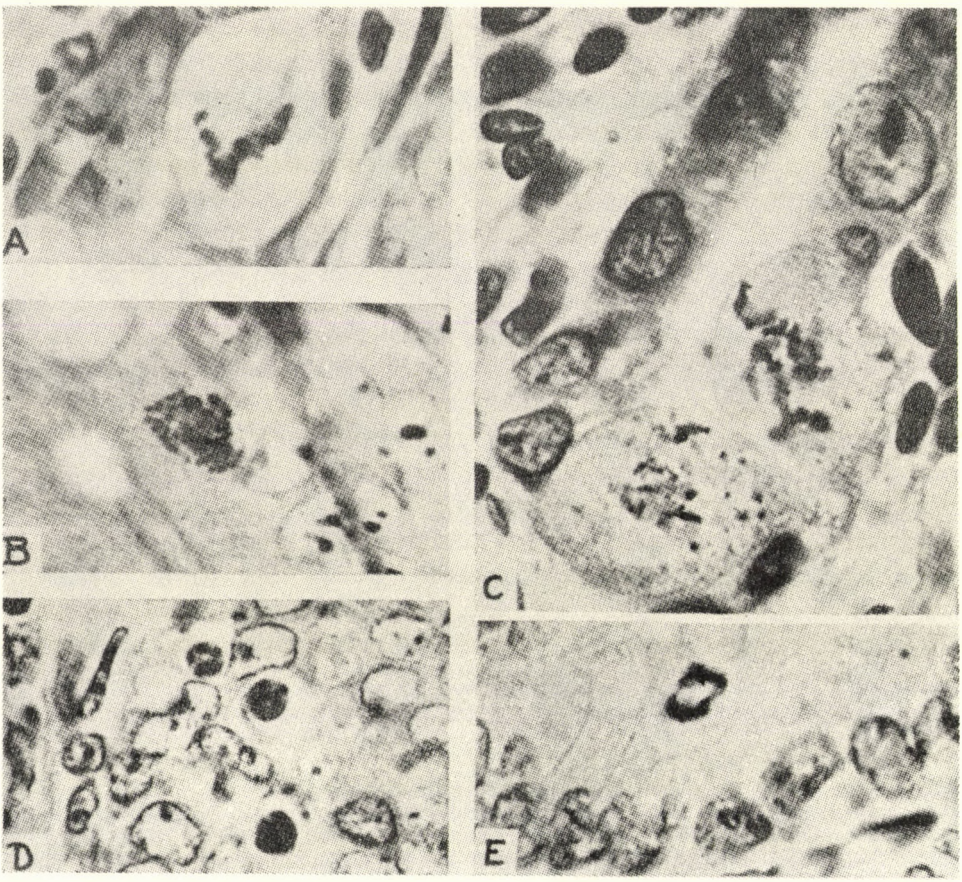


Fig. 1. Duodenum. Pathologic divisions in the epithelium of crypts. *A:* Chromatin granules arranged in V-shape in light cells. Dose: 100 microgrammes per 100 g body weight daily, for 4 days. Rat killed 24 hours after the last dose. H. E. stain. x1600. *B:* Fine chromatin granules and spherules at the place of the nucleus. No nuclear membrane, foamy protoplasma. The same rat. Heidenhain's stain. x1600. *C:* Dissipation of chromatin granules in a swollen, foamy cell, fine filamentous chromatin in another cell. Markedly swollen epithelial cells. Right top, nucleus inflated, sharp nuclear membrane, swollen nucleolus. Dose, 2×200 microgrammes per 100 g, killed 2 hours after 2nd dose. H. E. x1600. *D:* Rat, after 6 hours, peak effect. All epithelia cells foamy, sharply contoured nuclear membrane, swollen nucleoli. In 3 cells the cytoplasm has been replaced by vacuoles in which 1, 2, 3, 5, respectively, chromatin globules have been formed and surrounded by a thin border of cytoplasm. Close by, just visible round or slightly flattened chromatin globules. Dose, 100 microgrammes per 100 g killed 6 hours later. H. E. x1600. —*E:* Crypts filled with moderately swollen epithelial cells, a distorted diaster shifting toward the lumen. 1 dose of 100 microgrammes per 100 g. Killed 6 hours later. Feulgen's r. x1600

large, the cell soon disintegrates and the expelled nuclear substance becomes surrounded by a margin consisting of cytoplasm. All chromatoid particles are markedly stained by Feulgen's reagent.

Apart from the pathological patterns of division described, cells may be frequently observed in which the chromatin has taken a diaster form with irregular chromatoid bridges between the asterisks. Not infrequently these nuclei display a rhomboid shape (Fig. 1 E). The nuclear changes and the dis-

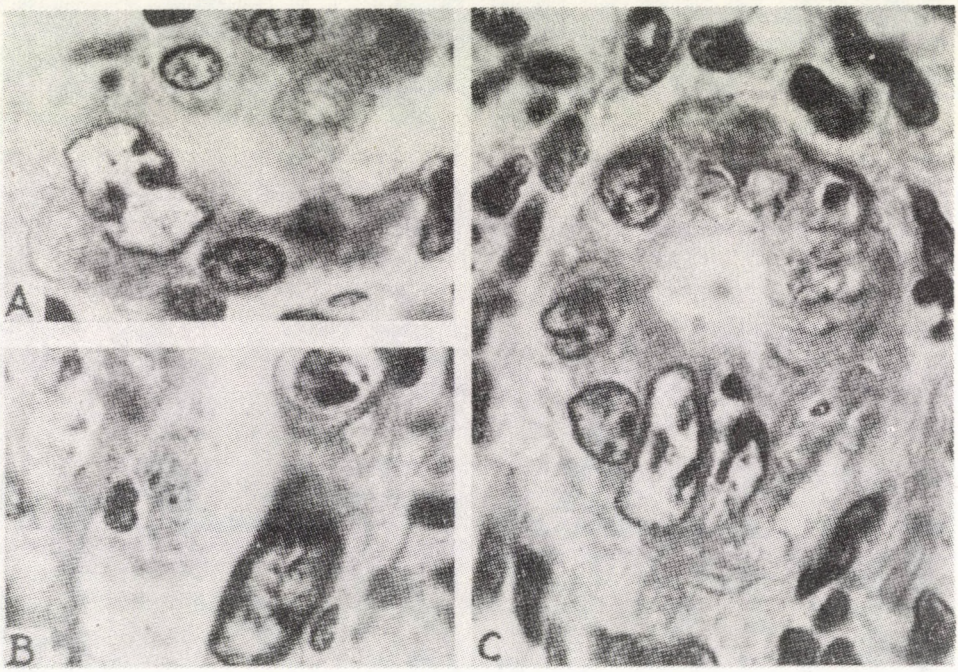
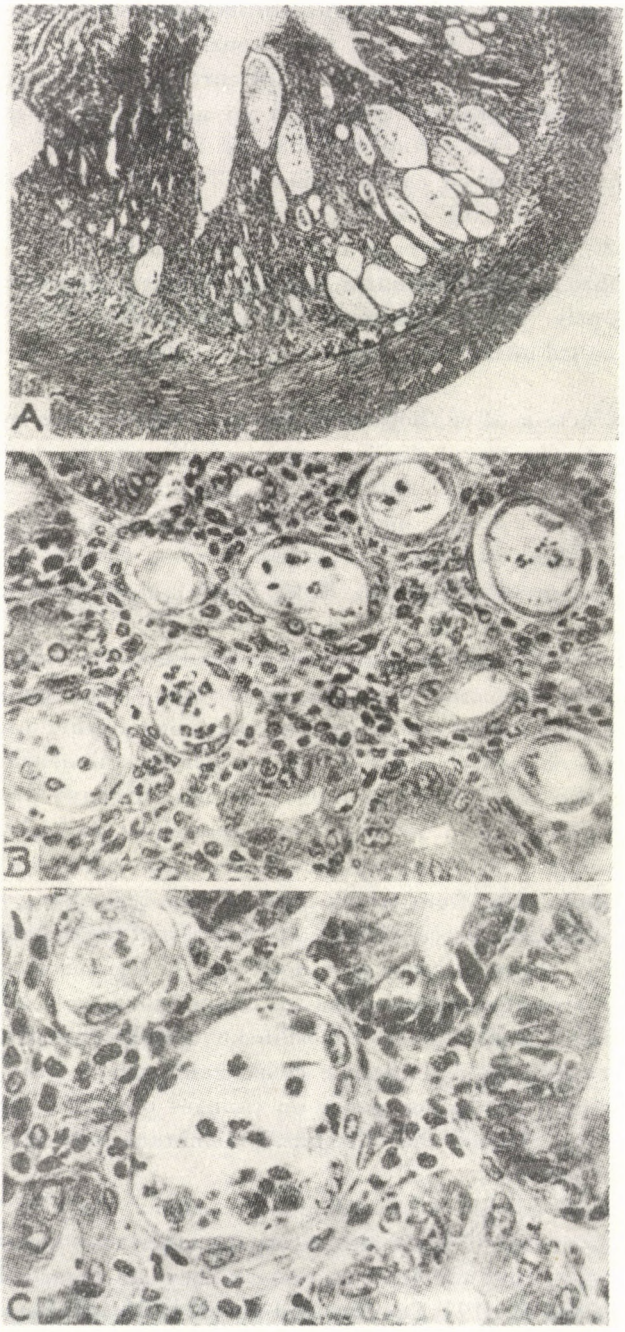


Fig. 2. 2 hours after the injection. Crypts lined with cells containing swollen and foamy nuclei, sharp nuclear membrane, coarse chromatin granules. In places recognizable nucleolus, otherwise obscured by chromatin. Destroyed cells and chromatin globules expelled toward the lumen. Right top, nucleus replaced by a vacuole, chromatin globule surrounded by thin cytoplasm entering into the lumen. Dose : 2 doses of 200 microgrammes per 100 g each. Killed 2 hours after the second dose. H. E. x1600

sociation of chromatin resembles the picture produced by colchicine, except that formation of powder-like globules was never observed after colchicin treatment. The division form resembling a diaster is also characteristic of nitrogen mustard action.

On account of the extensive destruction, the structure of the intestinal mucosa is deeply changed at the bottom of the crypts where there hardly remains a single undamaged cell. Changes similarly grave naturally do not occur except after high doses. Although the injection of nitrogen mustard is



A: Dosage, 75 microgrammes of nitrogen mustard per 100 g of body weight twice a week for 2 months. Killed 48 hours after the last dose. Body weight about 140 g, with little fluctuations. H. E. stain. Low power

B: Treatment for 6 weeks with the same doses. Killed 6 hours after the last dose. Body weight from 125 to 130 g. H. E. x520.

C: The same section x830

Fig. 3. **Duodenum. Cystic dilated crypts in animals exposed to** chronic treatment. Crypts lined with basal membrane or new flat epithelium. In the lumina desquamated epithelial cells and chromatin granules formed after their decomposition. Extensive round cell infiltration in the interstitium.

followed within 1 hour by the formation of numerous chromatin globules the most severe changes occur between the 6th and 12th hour. Thereafter the changes are gradually improving. Initially, the formation of chromatoid powder and the meshwork prevail, the decomposition of chromatin to globules becomes prominent later on. This would point to a certain order in the nuclear changes.

These structures cannot be observed except when cells in the process of mitosis are destructed. Cells exhibiting no sign of division are, however, also markedly injured (Fig. 2.). They have pale and enlarged nuclei, with a sharply outlined nuclear membrane. The chromatin appears in coarse irregular masses which stain homogenously. The cytoplasm is finely granulated. These cells, being probably in the so-called interphase, are very apt to undergo destruction (Fig. 2 B).

Once the extensive destruction of epithelium in the crypts has come to an end, regeneration is started. The morphological course of this process can best be followed in animals which have suffered a chronic poisoning, although it may equally be observable after a single, higher or lower, dose of nitrogen mustard. In the duodenum, colon, and small intestine, lumina undergoing cystic dilatation are found in the mucosa (Fig. 3 A, B, C). Some lumina contain only chromatin globules surrounded by a thin cytoplasm border. The epithelial lining has been extensively destructed and in numerous places the basal membrane is seen. In other lumina, regeneration of the epithelium has already started. Initially, only in some sections of the lumen has the lining epithelium become flattened. The cells are characterized by a flat cytoplasm and nearly normal-sized nuclei which make the cytoplasm bulge. Mitotic divisions are rarely seen. Sometimes the entire lumen is lined with epithelium gradually increasing in height, and developing first into cubicoid, later into cylindrical cells (Fig. 3 B, C). It is not quite clear from which kind of cells regeneration is started. Under normal conditions, the process begins at the basis of the crypts (*A. Schaffer*, [47] *Bizzozero* [5]). In the present case, however, the regenerating tissue seemed to spread downwards starting from the tip of the villi spared by the process. A further possibility, known from Lepeshinskaya's experiments, that the globules reorganize themselves and become cells again, must neither be omitted, as it has been pointed out in our paper on changes produced by colchicine. Finally, *Törő* [55] has claimed that the basal membrane may also be transformed into epithelial cells.

Liver. Our experiments with colchicine have demonstrated that the effect of the so-called mitotic poisons manifests itself also in organs other than those having a high mitosis index. As a rule, the liver and the kidney are regarded as indifferent structures (*Graef* and al. [19]; *Landing* and al. [37]; *Malyughina*, [41]; *Barberio*, [3]), although a low number of mitoses may be observed also in these organs. The action of nitrogen mustards manifests itself in the liver at an early period and in a very characteristic manner. *Barely* 10 minutes

after the injection, the endothelial cells of the sinuses become swollen and detached and appear free in the sinus in the form of round structures. This process must be considered as a toxic effect. Subsequently, the changes of the sinus endothelium become more characteristic and resemble those observed in the duodenum, as far as the chromatin is powder-like, the chromatin substance of cells the nucleus of which has undergone karyokinetic division is gradually precipitated (Fig. 5 E, F), chromatin globules surrounded by a thin ring of cytoplasm are formed and, finally, the sinus endothelium disintegrates (Fig. 5 G, H). Dissociation of cells which are in the so-called interphase may similarly

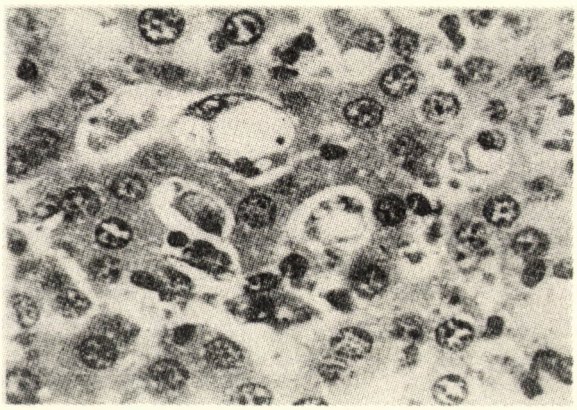


Fig. 4. Liver. Varying size of the nuclei of liver cells. Foamy Kupffer's cells, massive desquamation, nuclei pycnotic, distorted or vacuolized, foamy. Dosage, 100 microgrammes per 100 g body weight, repeated in the first week. Killed 72 hours after the second injection. H. E. x75 0

be observed. These cells are enlarged, they contain vacuoles beside a nucleus displaced to one side. Numerous endothelial cells exhibit phagocytized nuclear fragments in the cytoplasm. The fragments probably originate from disintegrated endothelium and liver cells. (The dysfunctioning of the hepatic reticuloendothelial system after administration of colchicine has been demonstrated by the quantitative determination of silver accumulation; *Sugár*, *Velősy*, [53]).

No marked liver injury was produced by a single dose of nitrogen mustard. On the other hand, repeated administration of the substance was followed in the few mitotic liver cells by characteristic changes essentially similar to the epithelial lesion observed in the intestine. The process can be easily followed in Figs. 5 A, B, C, D.

Another sign indicative of hepatic lesion is the appearance of fine fat droplets, further the focal necrosis observed in a few animals. The latter change, however, cannot be considered characteristic. In our opinion, differences in the size of nuclei are more significant. Beside very small liver cell nuclei, numerous very large mononuclear cells and polynuclear giant cells of hepatic origin may

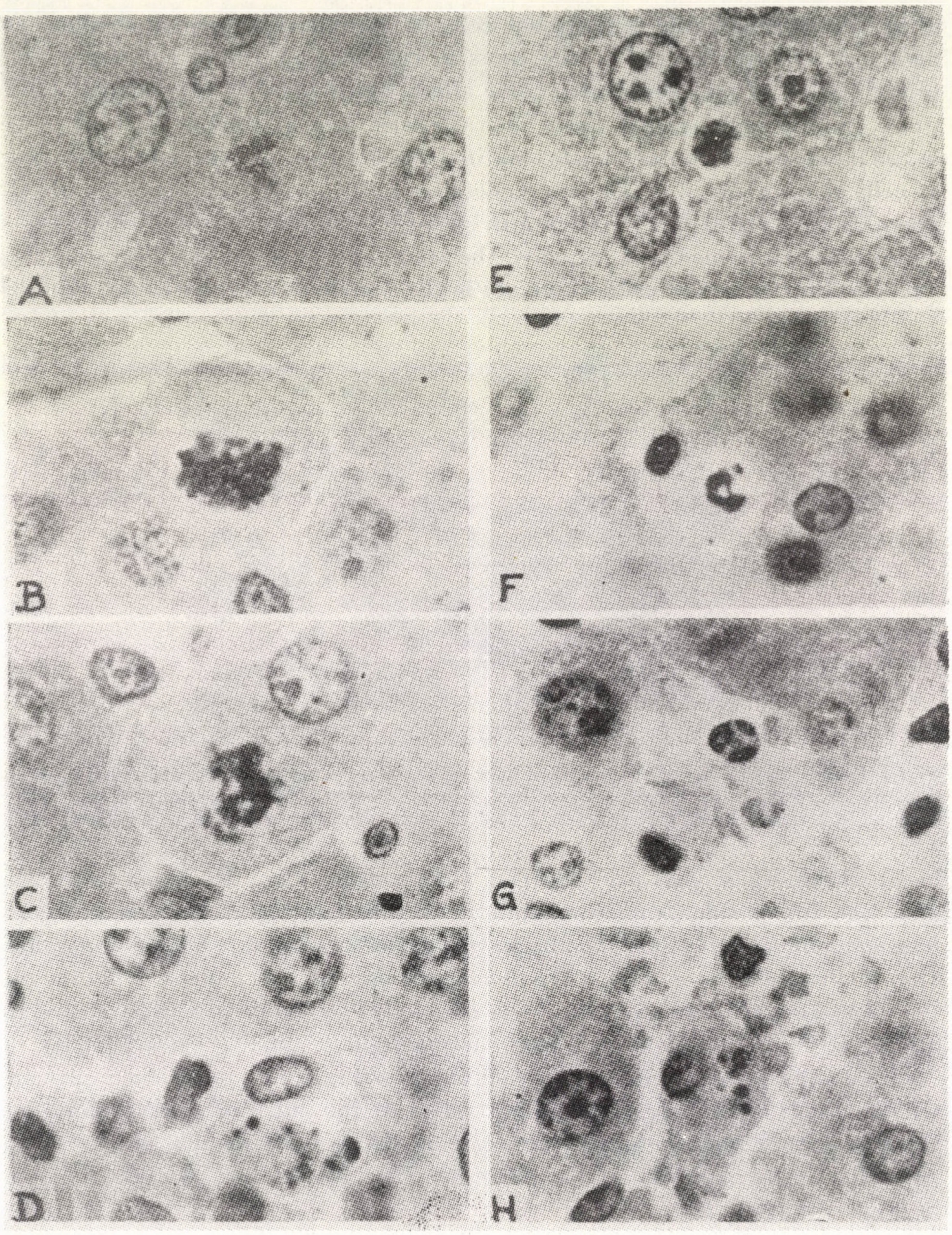


Fig. 5. Liver. *A*, *B*, *C*: Disorders of division in cells of parenchyma. *D:* Decomposing endothelia or parenchyma cell, chromatin in granules or globules. The changes are identical with those seen in the duodenal epithelium (Fig. 1.). Dosage, 4×100 microgrammes per 100 g (accumulated treatment). Killed 24 hours after the last dose. H. E. x1600. —*E*, *F*, *G:* similar nuclear changes in desquamating sinus endothelium. *G*: Liver cell with blurred outlines and disintegrated into nuclear spheres. *H:* Phagocytized chromatin globules in the plasma of an endothelial cell. Dosage, 1×200 microgrammes of nitrogen mustard per 100 g. Killed after 12 hours. H. E. x1600

be seen. Changes in the arrangement of chromatin, a sharply limited nuclear membrane, more than two nucleoli, and a light chromatin meshwork are rather frequent phenomena. Granular decomposition of the nucleus also occurs in a number of liver cells (Fig. 5 C).

Graef and al. [19] have found that mobilisation and excretion of liver glycogen occurs after the administration of nitrogen mustard. Adopting this result, the glycogen content of the liver has not been examined after single doses. In the rats exposed to prolonged administration of the drug, an augmentation of the glycogen content was observed 6, 24, 48, 72 and 120 hours after the last injection.

Lymph nodes. It is well known that the effect of nitrogen mustard on malignant tumours is not uniform and that up to now the best results have been achieved in neoplastic diseases of the lymph nodes, such as lymposarcoma, reticuloendothelial sarcoma, leukosis, Hodgkin's disease. (*Larionov* [33], *Larionov*, *Kuzmina*, *Nykonova*, *Petrov*, *Tseli* [34], *Heilmeyer* [21, 22], *Burchenal* [11], *Karnofsky* and al. [27], *Busse* [12], *Jacobson* and al. [25], *Graver* [15], *Arice-Kauter* [2], *Wintrobe* and *Huguley* [56], *Adler* [1]). From among Hungarian authors, *Sellei* and *Graf* [48], *Sipos*, *Jáksó* and *Szadeczky* [50], *Földvári* and *Nékám* [17] have made clinical observations with nitrogen mustard. Several authors (*Malyughina* [41], *Landing* and al. [37], *Graef* and al. [19], *De Bruyn* and *Robertson* [10]) stated that the administration of nitrogen mustard resulted in extensive disintegration in the lymph nodes, the weight of which became markedly decreased.

In the lymphatic system, pathologic forms of mitotic division occur much less frequently than in the duodenum and the liver. The powder-like chromatin structure, held characteristic of the initial phase, does not occur and only the second phase, the condensation of chromatin into globules of varying size and the destruction of cells, can be observed. Similarly to other cytotoxic substances, nitrogen mustard exerts its damaging effect mainly upon the follicles. The effect manifests itself in the 10th minute and after 30 minutes it is already marked. Within 1 hour the changes become very grave and after 6 hours they are fully developed. The cell destruction gradually extends to the margin of the follicles and by the 12th hour the entire substance of the lymph node exhibits signs of grave decomposition (Fig. 6 A, B). Beside the lymphoid elements, also the sinus displays profound changes, the endothelium becomes detached and occasionally undergoes destruction with nuclear decomposition. The Feulgen-positive chromatoid spheres originating from decomposed follicles are carried into the sinuses, all of which are widely dilated (Fig. 6 C and 7). The slightly eosinophil reticulum cells are loosened, become round and free (Fig. 7), many of them have undergone necrosis, with no visible nucleus or only 2 to 3 chromatin spheres instead of it, and with vacuoles in the cytoplasm. Despite the extensive destruction, loosened reticulum cells may be present for a long time, as a sign

of their constant re-formation. Numerous decomposing lymphoid elements may also be observed, the place of which is taken by plasmatocytes situated

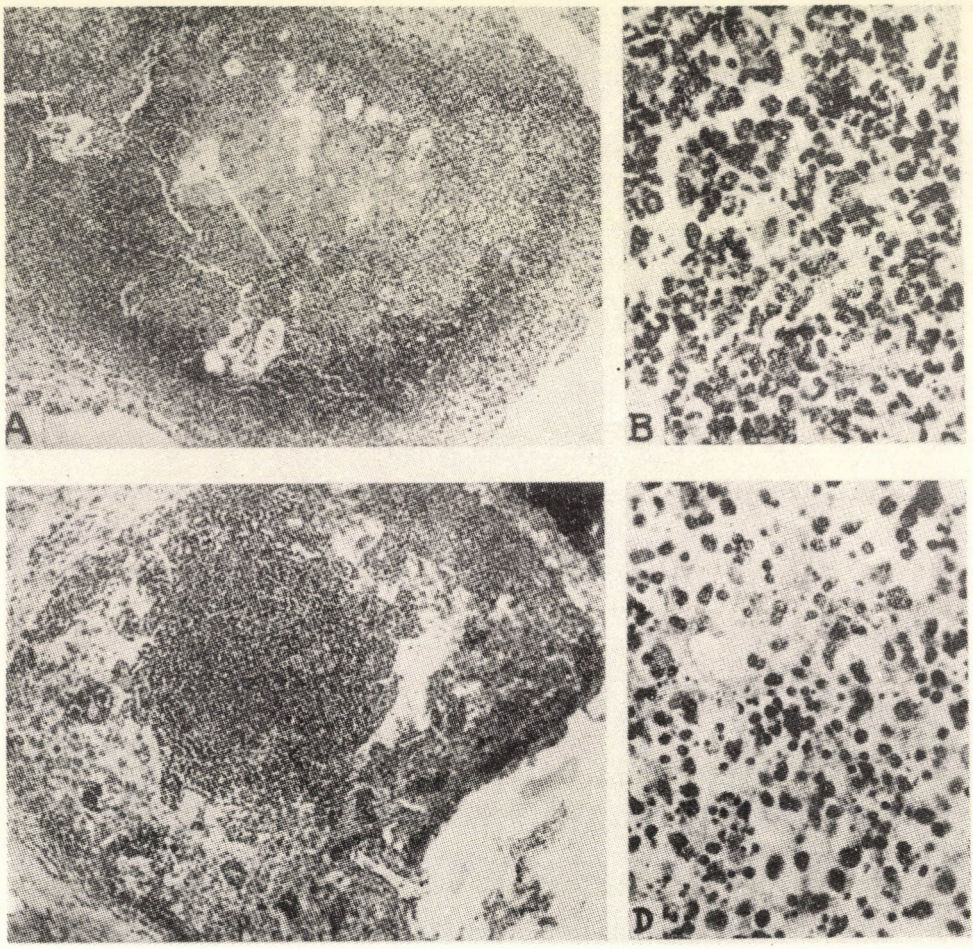


Fig. 6. A, B: Lymph node after acute treatment. Medulla near y empty, the cortical structure is hardly discernible on account of the granular decomposition of lymphoid cells *(B)*. Dosage 1 × 200 microgrammes per 100 g. Killed after 24 hours. H. E. x100 *(A)*, x 250 *(B)*. *C, D.* Lymph node after chronic treatment. Extremely dilated sinuses, extensive destruction of lymphoid cells, prevailing reticular elements. 4 months treatment with 75 microgrammes per 100 g once a week. Killed 72 hours after last dose. H. E. x 100 *(C)*, x 250 *(D)*

between the enlarged sinuses. Plasma cells show a greater resistance to nitrogen mustard than the follicles and lymphoid cells (Fig. 7).

When nitrogen mustard had been administered for 2, 4 or 5 days, the entire lymph node underwent destruction, its cells became transformed to small chromatoid spherules, the original structure of the lymph node was unrecogniz-

able. In consequence of prolonged treatment, diffuse reticular hyperplasia occurred in the sinuses, the follicles, and the pulp (Fig. 6 C, D). The destruction process came to an end quite soon, after about 96 hours, but the round eosinophil reticular cells persisted in the sinuses for a long time afterwards.

The spleen becomes rapidly atrophized. On prolonged treatment the organ decreases in volume to hardly half or one third of the normal. The basic changes

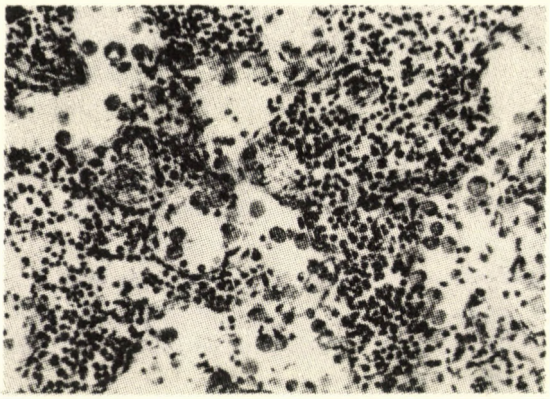


Fig. 7. **Lymph node. Dilated sinuses, desquamating endothelial and reticulum cells with eosinophil cytoplasm and disintegrated nuclei, as in Fig. 6. Lymphoid cells mostly destroyed and replaced by plasmocytes. Treatment with 4×100 microgrammes (accumulated treatment), killed 24 hours after last dose. H. E. x 280**

are quite similar to those occurring in the lymph nodes. The white pulp shows the same damage as the follicles of the lymph nodes. The Malpighian bodies soon diminish in size. Prolonged treatment results in the 6th to 8th week in a marked reticular hyperplasia, with the appearance of giant cells possessing several pale nuclei. Both single doses and prolonged treatment are followed by disappearance of cells from the pulp, dilatation of sinuses and desquamation of the sinus endothelium and subsequently by fibrous transformation of the reticulum.

A dose producing severe changes in the intestinal mucosa (100 microgrammes per 100 g body weight) has, in the 6th hour after the injection, hardly any sequel in the spleen. If this dose is repeated for several subsequent days, grave changes appear also in the spleen.

The thymus becomes equally atrophic. In animals which had been exposed to chronic treatment, the gland can hardly be found. The thymocytes are destructed simultaneously with the lymphocytes. The elements destroyed are carried from the cortex into the medulla which latter therefore seems to be more rich in cells than the cortex until 12 or 24 hours later when the thymocytes have been finally eliminated from the medulla where only the reticulum remains. These observations are in accordance with those of *Graef* et al. [19].

All cells destroyed or set free in lymphatic organs are being carried away by the lymph stream. This is, why these organs become reduced in volume. After the parenchymatous elements have disappeared the stroma becomes prominent. The form elements carried away can often be demonstrated in the cortical sinus of a lymph node belonging to the next level.

The alterations of the *testicles* were studied in mice by *Landing* et al. [36] with quantitative evaluation of the damaged cells. Their results have been corroborated by our observations of which it may therefore suffice to give a brief summary. Spermiocytes incurred the most severe damage. The evaluation of testicular changes in this organ presents quite a problem as the spermiocytes display much variety also in control animals. The chromatin substance becomes more disperse and the nuclei do not stain. In the layer of spermiocytes high numbers of chromatin globules appear. These, together with the chromatoid granules which measure 1 to 2 micra and possess a thin protoplasm border, are frequently found in the lumina of the testicular tubules, beside immature forms. After administration of colchicine or podophyllin, a great number of giant cells derived from spermiocytes or spermatides were seen to appear. In animals receiving prolonged nitrogen mustard treatment, giant cells were found only exceptionally. The dysfunction of spermiogenesis varies even within one testicle, being marked in some canalicular sections whilst in other areas a mass of mature spermia are present. In rats exposed to chronic treatment, the lesion of spermiogenesis is associated with a damage to spermio-histogenesis. In many testicular canals no mature spermia are formed in the epididymal tubules, only debris originating from the destruction of immature forms, chromatin granules of varying size and intact spermiocytes may be seen. In a few animals the spermio-histogenesis remained unimpaired. We were unable to determine the precise time at which restoration occurred in the animals treated with single doses, changes provoked by such treatment being neither well-marked nor characteristic. Following chronic administration, full restoration takes about a fortnight. (According to *Landing*, the effect is of a longer duration, lasting from 3 to 4 weeks.)

Changes in the *lungs* resemble those induced by colchicine. In the septa a great number of destroyed cells may be seen, the origin of which cannot be established. Destroyed leucocytes and alveolar cells carried into the spaces of lung tissue may likewise occur, together with numerous siderophage cells and, strikingly, a few giant cells in the connective tissue. The epithelium of both the bronchi and bronchioli becomes detached and so does in some cases the alveolar epithelium.

Little can be said of the *kidney*. In the proximal convoluted tubules enlargement of the nuclei may be observed as soon as 20 minutes after the injection. Frequently, especially in case of cumulative dosage, large mononuclear cells are formed with enlarged disk-like nucleoli (Fig. 8). The variation curve of nuclei

of the proximal convoluted tubules shows that the nuclear diameter is markedly increased. (This fact will be dealt with in detail in another article.) The glomeruli are intact. A few cells of the tubular epithelium undergo pyknotic disintegration

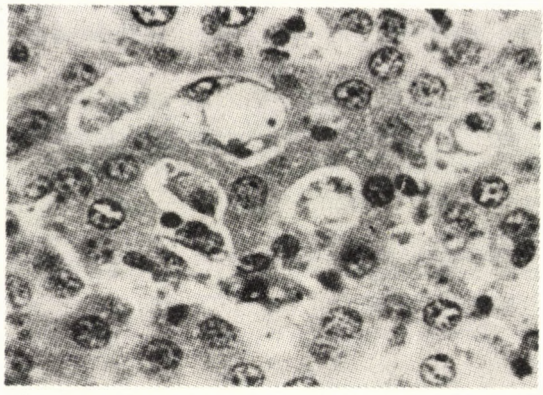


Fig. 8. **Kidney. Enlargement of nuclei in tubular epithelium, desquamating cells. Single** injection of 200 microgrammes per 100 g. Killed 20 min. after injection. H. E. x 830

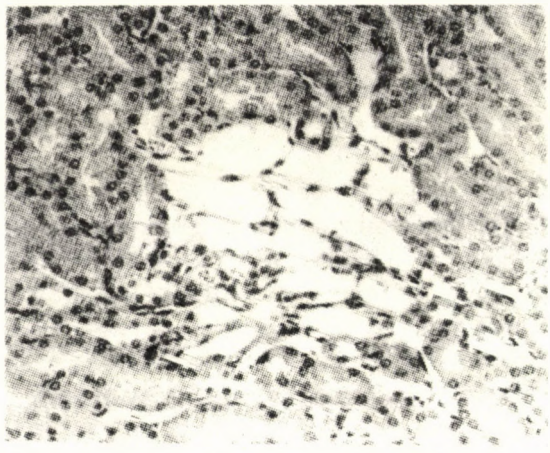


Fig. 9. **Kidney. After the expulsion of tubular epithelium the network of the basal** membrane constitutes a cystic structure. Below, regenerating epithelial proliferation like in the duodenum (Fig. 3.). Dose, 2×100 microgrammes per 100 g. Killed 72 hours after the second dose. H. E. x 250

and become detached and it may also occur that after the desquamation of the epithelium the network of the basal membrane is left back like a frame. Signs of epithelial regeneration are present like in the duodenum (Fig. 9).

The changes of the *lymph follicles* occurring in some organs (lungs, intestine) of the rat exhibit the same morphological features and course as the lymph nodes.

Comment

Several opinions have been formed concerning the action mechanism of nitrogen mustard, according to all of which the essential effect consists in inhibition of some enzymatic process. It is common belief that, following the ionisation of nitrogen mustard, a cross linkage is formed between protein and nucleoprotein molecules. The active group of nitrogen mustard, the bifunctional carbonyl-imino radical would enter into reaction either with enzymes containing a sulfhydril group, or with other groups, or perhaps it would be directly bound to desoxyribonucleinic or ribonucleinic acid. *Brand* and *Griffin* [9] could protect animals against the lethal effect of a high dose of nitrogen mustard by administering a great quantity of cystein, just as *Patt* and his associates [44] have averted the effect of X-rays. These facts support the assumption that the effect of nitrogen mustard is closely related to the sulfhydril group. *Gilman* and *Phillips* [18] claim that nitrogen mustard acts upon several enzymes, first of all on phosphokinase.

According to Selye, Dustin's karyoclastic crisis should be regarded as a stress. This view has not been adopted by *Karnofsky*, *Graef*, *Smith* [28]. The different progress of the effects of nitrogen mustard and colchicine may serve as a further argument in favour of the specificity of the action. The cytologic effect of nitrogen mustard is exerted on explantated tissue as well. (*Schreck*, [46]; *Tell*, *Alsop* [54]). Organs temporarily excluded from the blood circulation show no sign of injury after administration of nitrogen mustard (*Karnofsky* et al., [28]). These facts, in our opinion, suffice to prove that the changes are due to a characteristic poison effect.

Morphological examinations have revealed another aspect of nitrogen mustard action. On the basis of our results it seems warranted to refute the opinion of J. *Bichel* [4] and *Heilmayer* [21], according to whom the effect of nitrogen mustard would be limited to a certain region of the chromosomes, the so-called heterochromatin. Although the most marked lesions occur in the nuclei, the changes observable in the cytoplasm are by no means inconsiderable either. The close correlation between nuclear and cytoplasmatic nucleoproteins demonstrated by Lepeshinskaya makes it probable that simultaneously with nuclear injury the cytoplasm becomes equally injured.

Nitrogen mustard exerts its action not only on dividing forms but also on resting cells. It is for this reason that the effect of the compound has been termed »radiomimetic«. It has been supposed that only resting cells are damaged and that the pathologic arrangement of the chromatin of dividing cells would be due to a previous lesion (*J. Bichel*, [4]; *Loveless* and *Revell*, [39]; *Karnofsky*, [26]; *Madsen*, [40]; *Hohl* and *Schinz*, [23]). In contradistinction to this, colchicine is thought to damage exclusively the dividing cells. This form of distinction between the two compounds, both of which interfere with the

whole course of cell development, seems unjustified in spite of the fact that the most marked and especially the most characteristic morphological changes occur in dividing cells. As regards colchicine, the statement has more or less been proved that most of the dividing cells are found to be in the phase of metaphasis, on account of the fact that division cannot proceed beyond that phase and the majority of cells reaching the stage of anaphasis undergoes destruction. After administering nitrogen mustard no phase has been found to prevail among the distorted mitotic forms.

The most important difference between the effects of the two compounds was in the progress of the actions. The first changes produced by colchicine appear after one half to one hour, intensive cell destruction is observed after 2 hours, the peak of the effect occurs after 24 hours. After 48 hours, restoration is nearly complete and there hardly remains a sign of the toxic action suffered. The effect of nitrogen mustard appears much earlier. As soon as after 10 minutes changes are already observable and the nuclear injury becomes quite characteristic after 30 to 60 minutes. The peak of the effect is attained in 6 to 12 hours. It is another distinctive feature that, especially in the intestines, the effect of nitrogen mustard is protracted and decomposing nuclei may still be found as late as on the 5th day. This latter fact cannot be explained except by a recurrence of the destruction process. Very characteristic are the late changes appearing in the mucosa of the duodenum, ileum, and, less frequently, the colon.

Both compounds exert their action on all cells of the organism. The injury, however, appears in the single organs with fairly characteristic morphological features. From all the changes observed in poisoned rats, the duodenum seems the organ most suitable for estimating the effect. Within 6 to 12 hours after injecting the substance the changes induced in the duodenum become so profound as to render the structure of the crypts unrecognizable. Of course, the lesions depend both on the quantity of the drug administered and the frequency of the injections. A comparatively low dose may within 48 hours be foliowed by complete restitution. After a high dose and after repeated administration regeneration is protracted. Soon after the injection of nitrogen mustard, the disintegration of cells is started and the number of mitoses is lessened (»initial mitotic depression« of Werber et al. [57]). Simultaneously with the disintegration, grave reactive phenomena occur. The latter may be due to bacterial invasion in consequence of the epithelial destruction but it cannot be excluded that substances set free during decomposition are responsible for the inflammatory reaction. Part of the chromatin globules escapes through the lumina whilst the majority finds its way into the connective tissue surrounding the crypts. In surviving animals the epithelium of the intestinal mucosa soon becomes replaced while inflammatory phenomena may persist for from 9 to 13 days. Protracted administration has not been followed by permanent changes, aside from some cicatrization of the sub-mucosa. As mentioned above,

cystic dilatation of Lieberkühn's crypts is frequently present. During restitution the epithelium may be quite flat. This phenomenon has been taken by some authors for metaplasia (e. g. *Barberio* et al. [3] who observed it in the mucosa of the colon). It should be mentioned, in addition, that, according to *Griffin* [20], further *Boyland* and *Horring* [7], nitrogen mustard would promote neoplasm formation. In our view, decomposition and tumour formation are processes so closely related that the capacity of nitrogen mustard of giving rise to malignant growth may readily be explained, though such an effect did not occur in the course of our experiments.

The changes observed in the stomach and the entire alimentary tract were essentially identical. In respect of the severity of changes, the small intestine comes next after the duodenum, then follow the colon and the stomach where processes are gradually milder. Intestinal clinical symptoms have not been observed after nitrogen mustard administration (*Spitz*, [52]; *Malyughina*, [41]).

The lymph apparatus being less sensitive than the alimentary canal, the transitory reticular hyperplasia may be followed by complete restitution. The most conspicuous changes appear in the thymus gland, while those of the spleen and the lymph nodes are, as regards duration and quality, essentially similar.

The general effect exerted on the organism is well shown by the characteristic changes of the cells in the liver and the kidney.

Several authors have investigated the action of the compound on the haemopoietic apparatus (*Courtice*, [14]; *Cameron* and *Courtice*, [13]; *Bloch*, *Spurr*, *Jacobson* and *Smith*, [6]). The lesion of haemopoiesis is outside our concern. According to all the literary data, the effect is early and marked. The disturbance of spermiogenesis manifests itself at a later time and lasts longer, being, however, sooner or later, followed by restoration.

The differences between the changes occurring in various organs cannot be explained by the distribution of nitrogen mustard in the organism. Strikingly, the compound is not accumulated in organs displaying a high mitotic index, although it is in those organs that the most grave changes occur. *Skipper*, *Bennet* and *Langham* [51] have examined the distribution of nitrogen mustard in the organism by administering radioactive carbon (C^{14}). 6 hours after the injection the largest quantities were found, beside the lymph apparatus, the intestines and the testicles, in the brain and the adrenal glands. After 24 hours the greatest accumulation was found to be present in the adrenals and the thymus. Only little was bound by the bone marrow in which the maximum effect can be observed. The same results were obtained by *Boursnell* [8] who examined the distribution of sulphur-mustard containing radioactive sulphur. Similar examinations were performed by *Seligmann*, *Rutenburg* and *Friedmann* [49] by substituting the chlorine of nitrogen mustard with radioactive

iodine. They found that the tumour did not collect more radioactive substance than did the other tissues.

In our experiments, when the administration of a single high dose of nitrogen mustard had been repeated three or four times (accumulated dosage), the effect induced was, as could be judged from the histological changes and the course, nearly identical with the one observed after injecting one single high dose. Administration continued for several weeks or months was not followed by lasting changes either; the epithelium regenerated, the cystic dilatations of the intestinal epithelium vanished and the slightly increased amount of connective tissue remained the only lasting sign pointing to previous damage. Observing changes consecutive to prolonged treatment after periods identical with those after which the acute effect had been examined, it could be established that the lesions developed in such an order and form as if only a single dose would have been injected. All this corroborates the view that the chronic effect consists in nothing else but a repetition of several acute processes each of them induced by a single dose. The effect of nitrogen mustard lasts longer than that of colchicine. Thus one could assume that minute doses, administered during a longer period, cause the same effect as a large single dose.

The action of both nitrogen mustard and colchicine was examined in detail also on transplanted Guérin's cancer. The results of these examinations have already been reported at the 1st Congress of Oncology but it must be emphasized again that the cytologic changes observed in tumours were identical with those observed in organs. *Rapaport* [45] performed morphological examinations on human prostata cancers treated with synoestrol and stated that ». . .by the restitution of a tumorous disease the decrease of the tumour in size should be meant . . . occasionally its complete disappearance without the occurrence of destructive processes«. He emphasized, in addition, that decomposition should not be employed as a measure in the morphological evaluation of regressive processes. In his view, decomposition has a causal relation to growth and represents a cycle of the latter. Atrophy is the most reliable sign of tumour regression. The correlation of dystrophic phenomena with wound healing and tumour growth was already pointed out by us *(Dèvényi, Kellner*, 16; *Kellner, Holczinger*, [31]; *Kellner*, 29, 30). It is possible that hormone treatment exerts its effect upon tumours by means of atrophizing processes, perhaps through an amino acid deficiency. Dystrophic processes are, however, increased by both colchicine and nitrogen mustard, by which action the cell destruction caused by these compounds becomes considerably more extensive than the process necessarily presenting itself during tumour development. Both the compounds produce forms of cell disintegration never observed without their application.

Recording of dystrophic changes in a tumour is no easy task, especially not in transplanted tumours characterized by constant and extensive destruc-

tion. A method should be devised that would offer means for the evaluation of the cytotoxic effect in the presence of the constant and extensive dystrophic processes. The study of normal organs and tissues may be of assistance just in the understanding and evaluation of the changes occurring in tumours.

Summary

The effect of nitrogen mustard, methyl bis-β-chloroethylamine hydrochloride, was examined in 60 white rats. Histologic changes of the stomach, intestinal mucosa, lymph apparatus, liver, lungs and kidneys, were chiefly studied. Three forms of dosage were employed (i) one single dose of 100 or 200 microgrammes per 100 g body weight, (ii) the same dose given daily for 3 or 4 consecutive days, and (iii) 75 to 100 microgrammes per 100 g for from 7 to 155 days.

Nitrogen mustard damages not only cells engaged in the process of mitosis. Lesions were present in addition to those in the liver, kidney and lungs, also in every non-dividing cell of all the organs examined. This fact shows that the compound acts on the nucleoprotein substance of the entire organism.

The most severe changes occurred in the duodenal mucosa ; those of the small intestine and colon were, however, also marked and essentially similar. The lymph apparatus is almost equally sensitive. In the latter system the chromatin dissociated into globules while in the epithelium decomposition through mitosis was also frequent. In the testicles, injury to spermiogenesis prevailed.

Cell destruction started 10 minutes after administration of the drug. After 1 hour the nuclear substance became powder-like, subsequently to agglutinate into coarse globules while the cells underwent destruction. Diaster and other distorted pathological forms could be observed The maximum effect occurred after 6 to 12 hours. Intensive restitution began after 24 to 48 hours, remnants of the changes were, however, still present after 4 or 5 days, completely to disappear at a later time.

The chronic effect corresponds to repeated acute effects. Each single administration of nitrogen mustard is followed by identical changes which take the same course as those induced by a single injection.

A slight difference exists between the action of nitrogen mustard and colchicine, mainly at the onset. The changes due to colchicine develop at a slower pace and their regression is more rapid.

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ВЛИЯНИЕ ОСТРОГО И ХРОНИЧЕСКОГО ЛЕЧЕНИЯ ГОРЧИЧНЫМ АЗОТОМ НА ОРГАНЫ КРЫС

Я. Шугар и Б. Келльнер

Резюме

У 60 белых крыс мы исследовали гистологические изменения, вызванные лечением горчичным азотом (Г. А.) (Метил-бис хлористоводородный [хлорэтил]-амин), в первую очередь цитологические изменения, возникавшие в слизистой желудочно-кишечного тракта, в лимфатической системе, в печени, в почках и в легких. Г. А. подавался отчасти в большой дозе (200 гамма на 100 г веса), отчасти половиной этой дозы 3—4 дня подряд.

Следующая группа животных получила 75—100 гамма через 3 дня, в течение 7—155 дней. Животные убились в разных сроках после последнего впрыскивания.

Из исследованных нами органов самые тяжелые изменения встречались в слизистой двенадцатиперстной кишки, в которой уже 10 минут после подачи средства наблюдаются явления распада клеток ; через час вещество ядер распадает на мелкие пыловидные шарики, затем снова объединяется в более крупные глыбы, а клетки распадают. Можно видеть и патологические диастры, но и такие уродливые формы деления, которые

не напоминают ни на одну известную форму клеточного деления. Это говорит за то, что данное вещество оказывает до конца вредное влияние на клетки, находящиеся в делении. На органах максимум изменений наблюдается чаще всего через 6—12 часов; большинство изменений через 24—48 часов оканчивается, но возвращение к норме начинается в гораздо более медленных темпах, и следы изменений найдутся и 5 дней спустя. По сути дела подобные изменения наблюдаются в слизистой тонких и толстых кишек. На месте погибших клеток образуется новая популяция клеток; в лимфатических органах хроматин распадает на шарики; в яичке в первую очередь страдает спермиогенез. Влияние хронического действия, собственно говоря, соответствует повторенному острому действию потому, что каждый раз после инъекции развиваются такие же изменения, исход которых в общем такой же, как у острого действия.

Горчичный азот оказывает вредное влияние не только на делящиеся клетки; нам удалось обнаружить изменения и в печени, почках, легких, хотя там митозы встречаются редко. Это говорит за то, что этот яд влияет на нуклеопротейный состав всего организма. Мы подробно сопоставили гистологические изменения, возникавшие под влиянием колхицина с теми, вызванными горчичным азотом. В морфологических картинах, вызванных этими двумя средствами, имеется тонкая разница, проявляющаяся прежде всего во влиянии на делящиеся клетки. Цитологическое действие горчичного азота в начале различается от действия колхицина. Изменения, вызванные колхицином, развиваются медленнее и быстрее проходят.

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