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Correlation and Clusteranalytic Studies about Histological Characteristics of the Colon-Rectum-Carcinoma*

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Summary. We compared eight different histological and cytological qualities of colon and rectum tumors from 78 autopsied patients who had been clinically studied and treated. From the obtained values we calculated the CHI-squares and converted them into PHI-correlation-coefficients. The matrix of the obtained correlation-coefficients served for the further studies of cluster-analysis. We related biochemical and biophysical information about tumor biology gathered from the literature with the histological qualities we detected in the routinely prepared sections of tumor. The calculations of the cluster-analyses pointed to the existence of relationships between cytological qualities of a tumor, its histological qualities, and clinical information about the patient. From the structure of the statistical relationschips of dependancy, however, a significant error may result from an inadvertent selection of tissue and from the histological techniques of staining and sectioning.

Key words: Colon-Rectum-Carcinoma — Histology — Cluster-Analysis.

Zusammenfassung. Anhand von 78 ausgewählten, klinisch untersuchten und therapierten Sektionsfällen wurden insgesamt 8 verschiedene histologische und zytologische Untersuchungsmerkmale einander gegenübergestellt, der Tafelwert nach X^2 berechnet und zum phi-Korrelationskoeffizienten transformiert. Diese Korrelationsmatrix diente weiteren clusteranalytischen Untersuchungen. Anhand der Literatur werden die biochemischen und biophysikalischen Kenntnisse der Tumorbiologie in ihrer Relevanz zum histologischen Routinepräparat diskutiert. Gleichzeitig weisen die clusteranalytischen Berechnungen auf Beziehungen zwischen zytologischen Merkmalen des Tumors, histologischen Kriterien und allgemeinen Angaben des Erkrankten hin. Jedoch kann anhand der formalen Struktur der statistischen Abhängigkeitsverhältnisse ein nicht zu vernachlässigender Fehler durch eine (unbeabsichtigte) äußere Selektion und die histologische Färbe- und Schneidetechnik wahrscheinlich gemacht werden.

Schlüsselwörter: Colon-Rectum-Carcinom—Histologie—Cluster-Analyse.

Introduction

A great number of cytological and cytofunctional connexions were observed during the last years on different malignomas. The methods, partly very largescale and difficult to understand for the outsider, are mostly borrowed from biophysical and biochemical research work. The results, necessarily subordinated to the methods, are of limited generalization value. Basically there is brought up the point how far these results are actually of more general significance (than in it's own restictes methodical field) and if concrete connexions may be realized or even verified under entirely different conditions. This aspect of the paper seems to be of fundamental nature.

* Dedicated with reverence to Professor Dr. Wilhelm Doerr on the occasion of his 60th birthday.

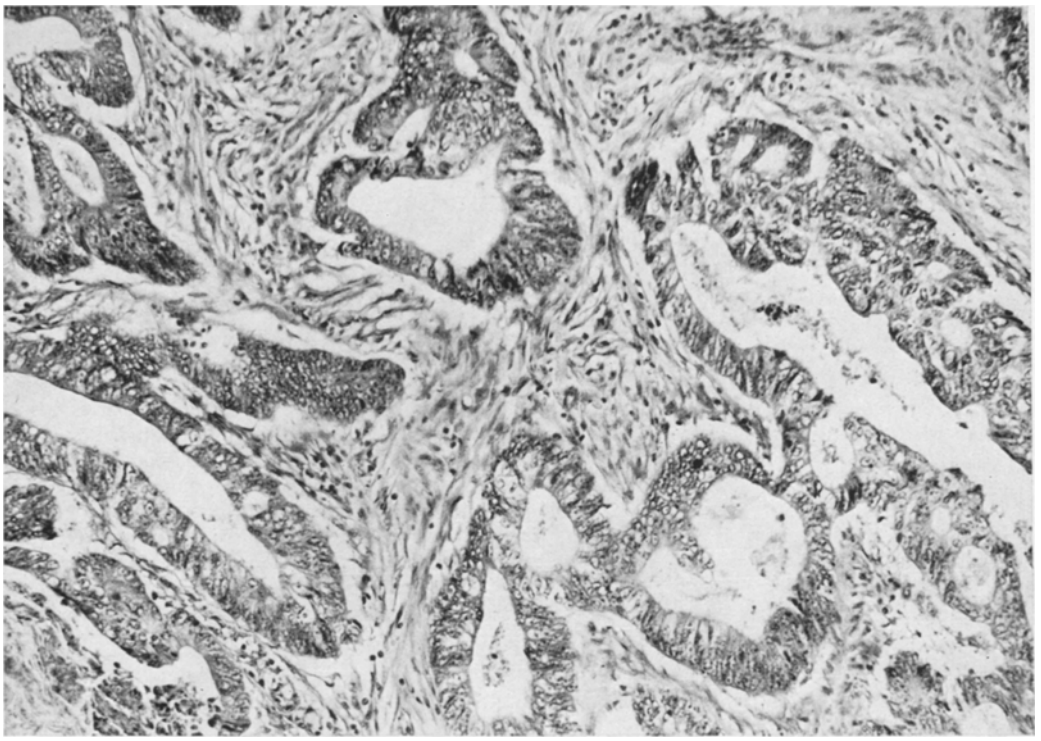

Fig. 1. B. 05975/64. Papillary Adenocarcinoma of the Colon descendens with lymphnode and peripheric metastases. Tumor fibrosis and lymphoplasmocytic infiltration, the latter specifically in marginal fields. Magn. 1:100

Another object is the pathoanatomic-histological preparation. The question is frequently raised, if in our days there may be gained more of it than a daily diagnosis. Elaborated, largescale and above all expensive methods are said to be necessary, more complicated structural connexions to be covered.

We suggest that one should pay more attention to the histological routine slide under the key-word "research applied to patients" and we put the attempt to reproduce patterns of most differenciated structural-morphological connexion with next to simple methodics into discussion.

Material and Methods

All cases of Colon-Rectum-Carcinoma ($n = 229$) with previous bioptic examination ($n = 78$) encounterd by autopsy during the years 1962–1972 at the Institut of Pathology of the University Heidelberg were evaluated. The examinated collective is composed of cases verfied by autopsy, existing already the clinical diagnosis Colon-Rectum-Carcinoma.

Apart from the general criterions of inquiry (as age of death, sex, localization of primary tumor, demonstration of local or distant metastases), 8 additional histological characteristics were investigated alternatively on their presence or absence (comp. Table 1: nucleoli, mitoses, shifting of nucleoplasmic ratio, mucous formation, fibrosis, lymphoplasmocytic infiltration as well as eosinophilic granulocytic infiltration and sinus histiocytosis of lymphnodes). By routine,

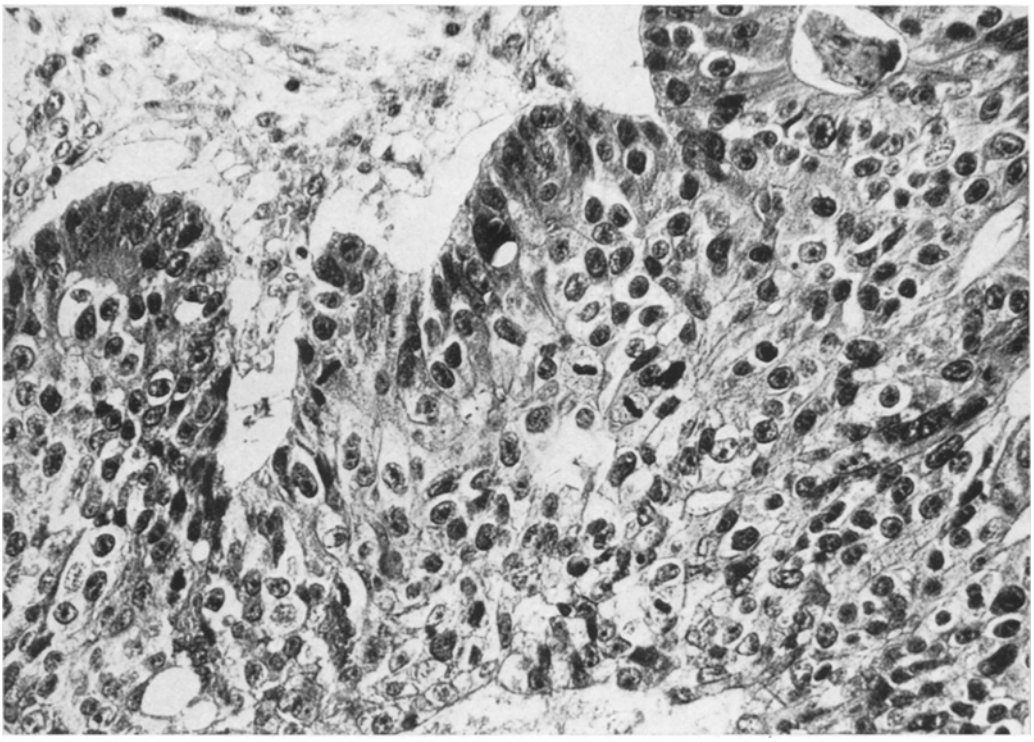

Fig. 2. Section of B. 05975/64 (Fig. 1). Characteristic numerous mitoses extending to the upper epithelial layers. Shifted nucleoplasmic ratio and some nuclei with large nucleoli are represented. Magn. 1:400

all the histological preparations were fixed in formalin and stained with haematoxylin and eosin. Only bioptic slides were examined (Fig.s. 1–3). In a few cases it was impossible to answer all the histological questions based on the bioptic slide. This leads the total number of examined cases to differ from $n = 78$ in some findings. The lists of findings were compiled in tables and then — separate for each pair of findings — opposed to each other in four-square tables (Sachs, 1968). The test was realized (if necessary after previous transformation in conformity with Freeman and Tukey, 1950) according to CHI-square with an alleged probability of error around $p \leqq 0.05$. The PHI-correlation coefficient could be calculated by the numerical value of each four-square table (Haseloff and Hofmann, 1970) (Table 1). Derived from this coefficient several clusteranalysis were supplied (McQuitty, 1957) (Fig. 4–6).

While the conventional and decision depending statistics may test only a pretended hypothesis which is compatible with chance (the "zero-hypothesis is accepted") or incompatible (the "zero-hypothesis is rejected", "there is a significant difference compared with the alleged theoretical variable of chance"), the clusteranalysis gives us more information. It is the concern of this method to rearrange a given matrix of correlation-coefficients (Table 1) on behalf of getting a more easily surveyable and entirely discussible structure of variables. A bunching of variables to in themselves homogeneous clusters is aspired for such a simplified structure. A coefficient of similarity (in this case the PHI-correlation-coefficient) applied to all pairs of variables is defined as a criterion of homogeneity (comp. Table 1, upper half). This coefficient should be as high as possible in a pair of variables which are both of the same cluster; the coefficient should be as low as possible if the variables of a pair are not of the same cluster.

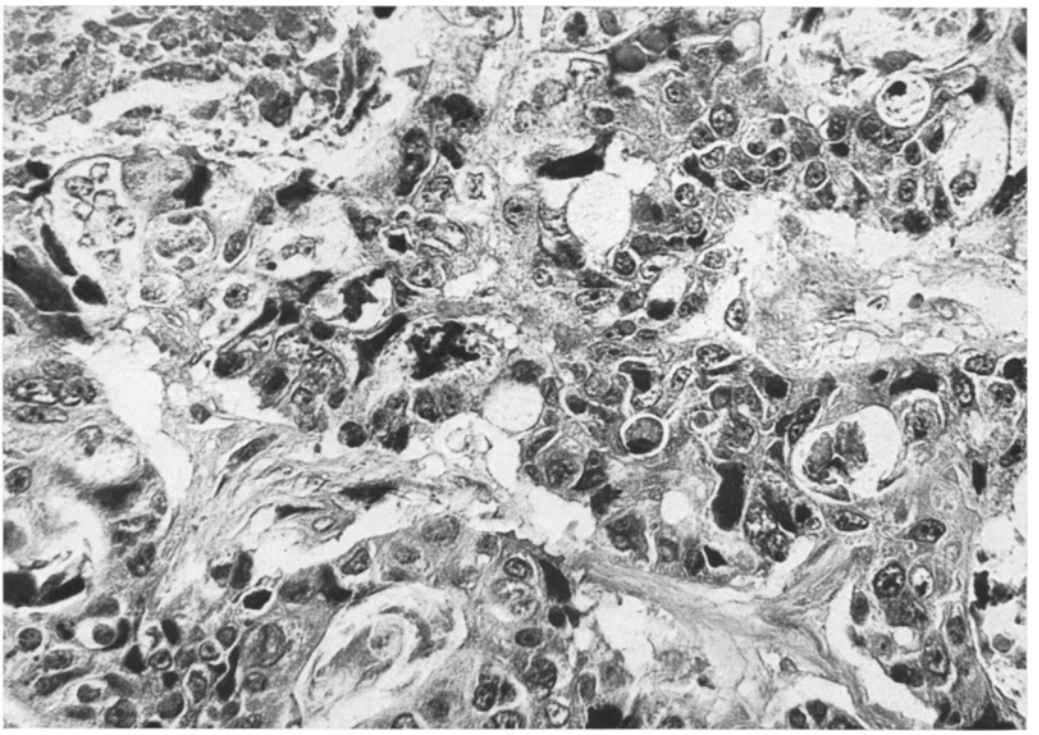

Fig. 3. B. 33772/71 (original slide), male, 36 y. Rectum Carcinoma. Numerous atypical mitoses and nuclear hyperchromatism are demonstrable. Magn. 1:500

The "linkage analysis" (Mc Quitty, 1957) is only able to show degrees of similarity in contrast to other proceedings, but this grading not only regarding the distance from a cluster to the cluster's nucleus, but also as a two-dimensional structure. We distinguish various similarity degrees in pairs of variables (comp. legend of Fig. 4):

1 very high similarity,
2 high similarity,
3 average similarity.

By formal description we differentiate chains (e.g. the variables 6–5–8–3 in Fig. 6), half crosses (comp. variables 1–7–2–4 in Fig. 4) and entire crosses as well as circles. The variables 5–6–11–7 (Fig. 4) represent a whole, with the relation between variables 6 and 7 even a half full circle. In Fig. 6 we find the variables 6–7–11 as a half circle. With the sequence from "chain" to "whole circle" (the latter does not exist in our calculations) the inside consistency of a substructure obtained formally in such a manner increases progressively.

Results and Discussion

In Table 1 are specified the results the results of the total of 66 calculated four-square tables. Among these, 12 table values were significant ($p \leqq 0.05$) and out of those 4 were highly significant ($p \leqq 0.001$). The average age of death (arithmetic medium) amounts to 64.8 years for men and 65.3 for women. A good agreement with the aftercare register values of the Surgical Hospital of the University Heidelberg (Bokelmann, 1972) was found regarding the age distribution too, as

Table 1. Correlation matrix of general and histological characteristics. The numbering refers to single variables and is identical with that of the cluster-analytic diagrams (Fig. 4–6). The CHI-square values of the foursquare tables are given in the lower half (left triangle), at which the number of * indicates the degree of significance: $p \leqq 0.05 = *$; $p \leqq 0.01 = **$; $p \leqq 0.001 = ***$. The arrows above the corresponding value indicate, together with the scale specirication, the direction of the table's positive correlation. Example: lymphoplasmocytic infiltration (10) and metastasizing (4) are significant by 4.36 ($p \leqq 0.05$); the arrow above the number shows to the top right, i.e. metastasizing "no" and lymphplasmocytic infiltration "yes" are correlated positively. — The PHI-correlation-coefficients calculated from the CHI-square table values are found in the upper half (right triangle)

		1	2	3	4	5	6	7	8	9	10	11	12
Age [-59 : 60-Y.]	1		0.198	0.062	0.335	0.037	0.086	0.446	0.083	0.079	0.083	0.020	0.222
Sex [♂ : ♀]	2	3.06		0.010	0.046	0.056	0.110	0.030	0.056	0.234	0.085	0.010	0.114
Localization [Sigma-Rectum; Coecum-Colon]	3	0.30	0.09		0.171	0.145	0.037	0.171	0.424	0.120	0.064	0.028	0.094
Metastasizing [yes : no]	4	8.77 **	0.16	0.48		0.048	0.038	0.107	0.049	0.100	0.236	0.119	0.166
Nucleoli [yes : no]	5	0.11	0.24	1.64	0.18		0.375	0.622	0.305	0.051	0.041	0.079	0.183
Mitoses [yes : no]	6	0.58	0.94	0.11	0.12	10.97 ***		0.2711	0.017	0.130	0.080	0.281	0.054
Nucleoplasmic ratio [shifted; not shifted]	7	10.73 **	0.06	2.28	0.90	30.2 ***	5.73 *		0.076	0.155	0.114	0.263	0.064
Mucous formation [yes : no]	8	0.54	0.24	14.04 ***	0.19	7.25 **	0.02	0.46		0.098	0.051	0.000	0.079
Tumor fibrosis [yes : no]	9	0.49	4.27 **	1.12	0.79	0.20	1.31	1.87	0.75		0.155	0.102	0.056
Lymphoplasmocytic Infiltration [yes : no]	10	0.54	0.57	0.32	4.36 *	0.13	0.50	1.01	0.20	1.87		0.627	0.127
Eosinophilia [yes : no]	11	0.03	0.05	0.06	1.11	0.48	6.15 *	5.38 *	0.00	0.82	30.64 ***		0.037
Sinushistiocytosis Lymphnodes [yes : no]	12	3.04	1.01	0.69	2.16	2.62	0.23	0.33	0.49	0.24	1.26	0.11	

well with the statements of Dukes (1958), Bacon (1964) and Shepherd (1971). The sex ratio (58% men, 42% women) is well in agreement with the value stated by Linder (1971) and Bokelmann (1972). However, some authors (Higginson, 1966; Wynder and Shigamatsu, 1967; Shepherd and Jones, 1971; De Jong *et al.*, 1972) mention a sex ration which seems to be shifted slightly in favour of women. Of course not always it may be clearly gathered if the statements refer to the age of falling ill or to the death age and on which populations the investigations are based. Greater differences are found regarding the distribution of frequency of diverse localizations, at which the Rectum-Carcinoma predominates largely in all clinical statistics, compared to the Colon-Carcinoma. In our autopsic material only 44% of the total cases of Colon-Carcinoma were of rectal origin, in comparison

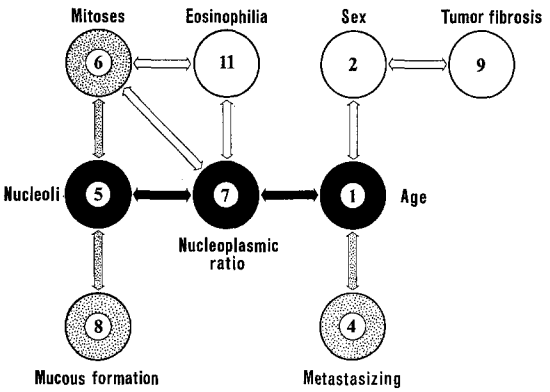

Fig. 4. Cluster I. The number in the round fields stand for the variables (comp. Table 1). Black fields characterize a variable with a very high similarity ratio (black arrows) to at least one additional variable. Dotted fields and arrows indicate a high similarity, white fields and arrows a low one. In this cluster 9 of the total 12 variables are represented; the variables 1, 2 and 9 are found only here

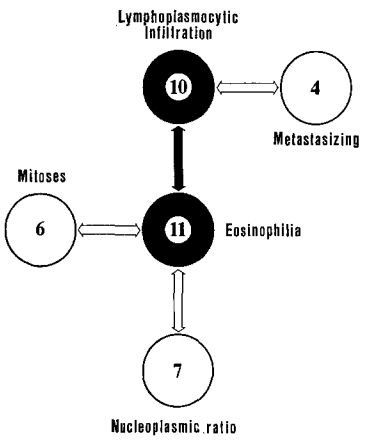

Fig. 5. Cluster II. This cluster has all the variables, except number 10, in common with cluster I, but with cluster III only the variables 6, 7 and 11. The variable 10 is represented only in this cluster

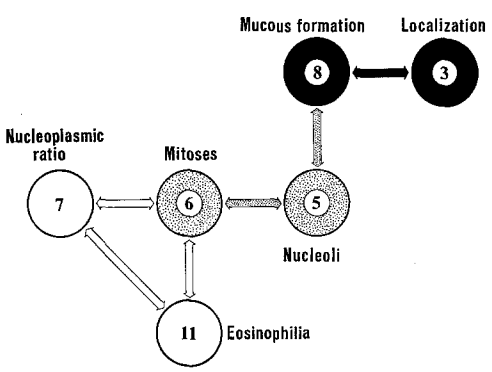

Fig. 6. Cluster III. The variable 3 is found only in this cluster; the variables 5, 7 and 11 are represented also in cluster II — the variables 5, 6, 7, 8 and 11 also in cluster I

with 61.3% of Bokelmann (1972), 52% of Shephard (1971) and 70.18% of Bacon (1964). A statistical comparison of these statements concerning all the localizations (a total of 6) gave no significant difference between the authors ($p \leqq 0.01$).

The dependence between metastasizing and age, analysed in our material, represents an important indication on the selection degree and on the generalization dignity of the results gained with this material. Metastasizing and age show a significat dependence so far as autopsy demonstrated more frequent metastases at lower age of death than at higher age. It is known that the frequency of autopsy declines with advancing age (Höpker, 1970). Bokelmann (1972) furthermore showed in the case of Colon-Rectum-Carcinoma, that elder patients die with increasing rarity on their carcinoma. The causes of death disseminate more and more with advancing age, so that elder patients finally succumb to other complications, but not to their already diagnosed and treated Colon-Carcinoma. From this results a determined selection of our material, such as to reduce for those patients with higher age the chance of succumbing to the already known carcinoma or to actually die in a hospital and latterly be autopsied. It remains to be seen if the deviation of the sex ratio might be explained by a determined external selection too. However, the significant value of the four-square table regarding metastasizing and age may be interpreted easily by a previous and in this sense determined selection.

We couldn't show more extensive processes of selection in the here presented material: the test values between age, sex ans localization were not significant. For the following investigations of histological and cytological characteristics we have therefore supposed independence of a determined external selection.

In the first instance the test results shall be discussed individually and then faced with the known bibliographical facts. Besides the age, a significant dependence ($p \leqq 0.05$) resulted by opposing the metastasizing and the demonstrated lymphoplasmocytic infiltration of the tumor. Tumors without lymphoplasmocytic infiltration showed no metastases after autopsy in only 8 cases, while 35 cases without metastases were observed in tumors with that infiltration. Though here could be effective a certain "external" selection too, we incline nevertheless to give certain credit to this result. It is intensely discussed since the experiments of Southam *et al.* (1966) and Brunschwieg *et al.* (1965) if a local, largely lymphocytic infiltration at the borders of malignant neoplasms might be interpreted as a cellular immunological protection. Southam could achieve an inhibiting effect of the growth-speed of tumor cells by application of lymphocytes. It is known for decades that lymphoplasmocytic infiltrations of considerable density occur in malignant neoplasms of colon and rectum as well as in other human organs and that they represent a favorable prognostic sign (McCarty, 1922; Mayr, 1953; Black *and col.*, 1954; Burkett *and col.*, 1956; Sherwin and Zovickian, 1961; Bagshaw, 1970). It is obvious why this phenomenon, which is performable by animal experiments too, is interpreted as a immunological defensive reaction in the sense of a "graft versus host-reaction" against the malignant tumor tissue, a defensive reaction which is certainly insufficient (Diamandopoulos, 1967; Old and Boyse, 1964; Woodruff, 1964; Lindholm and Rydberg, 1971). Presumably it is a question of a delayed reaction of hypersensitivity (Stewart, 1969). Experimental investigations on the Walker-tumor have shown that the plasmacells formed in spleen, lymphnodes and thymus may be detected in the tumor tissue and that comparatively

early. They are placed close to the tumor cells, at which they seem to be able to destroy them by almost clutching to them with delicate and finest branches of the cytoplasma (Hoepke, 1954). Also here, the biological task of plasma cells, whose first stage — the plasmoblasts — probably derive from the primitive large lymphocytes, seems to consist in originating, produce antibodies and die away (Steffen, 1968). Considering colon- and rectum-adenocarcinomas it is not exactly known if these antibodies turn against tumor-specific antigens, so above all against the carcinoembryonic antigens, or against products of tumor disintegration (Nagel and Geiger, 1971; Crowther and col., 1969).

Tumors of the gastrointestinal tract show not infrequently a marked infiltration of eosinophilic granulocytes in addition to lymphocytes and plasmacells. Corresponding investigations on the carcinoma of the portio cervicis uteri have shown that the appearance of eosinophiles improves the chances of healing (Schoch, 1926). They infiltrate in large numbers those tissues where antigen-antibody reactions are ocurring and seem to be attracted by products of this reaction, whose active principle couldn't yet be identified. The function of the eosinophilic granulocytes in this case is unknown (Humphrey and White, 1972). At least it permits the conclusion that, in connexion with an immunological tumor defense fixed to cells, the appearance of eosinophilic granulocytes must be related to the presence of immun-competent cells like plasmacells and lymphocytes. In this connexion we refer to the repeatedly observed hyperplasia of lymphnodes in the drainage areas of malignant tumors (Black, 1965). This is said to be related with a lower ratio of metastases. However, we did find no dependence of the lymphnode histiocytosis upon any of the mentioned general and histological findings. Nevertheless, in agreement with Broders (1920) and McCarty (1922) we consider it necessary to describe and inform also the "inflammatory component" in cases of tumor appraisement. The fact of observing less frequently an enlargement of the nucleoplasmic ratio in older patients than in younger ones could have to do with the age-depending decrease of regeneration of epidermal tissues, which on its part is connected with a general reduction of nuclear volumes (Tschahargane *et al.*, 1971). Possibly this age-dependent decreased nuclear hydratation is also effective in the tumor cells, so that at higher age a normal nucleoplasmic ratio is more frequently found in the tumor tissue.

The increased ocurrence of mitoses at increased nucleoplasmic ratio is a sign of decreased differentiation and of loss of control of DNA-synthesis. At that, the increased ocurrence of mitoses in the histological slide is not necessarily a sign of intensified proliferation, but by all probability due to a very retarded progress of the mitoses, since the frequency of observation of a cellular condition is a product of the cell number and the relative duration of the condition. Numerous observations support this assumption (Fabrikant, 1970; Hoffmann and Post, 1967; Lieb and Lisco, 1966; Steel and Bensted, 1965; Lipkin *et al.*, 1970). The cytoplasmatic functions of the cell are found predominantly during the G1-phase of the cellular cycle. A prolongation of the S- and G2-phase at the cost of the G1-phase therefore results in an increased nucleoplasmic ratio. Such changes of the cellular cycle were observed by Lipkin (1971) on Colon-Rectum-Carcinomas.

The nucleolus is considered as a control member of the cells' DNA-activity. According to Perry (1967) and Maden (1968) first stages of the ribosomes are

formed and stored in the nucleolus; the protein synthesis in the cytoplasma is controled by the releasing ratio. The proportions of nucleolus and nucleus keep constant within restricted bounds in normal cells (Ivanyi, 1971), while we observe a variation of size and shape of the nucleoli in neoplastic cells (Studzinski and Gierthy, 1972; Love and Walsh, 1970). It is possible to deduce from theses authors' observations that with an increased nucleoplasmic ratio, i.e. with increased activity of the chromatin, the formed first stages of ribosomes cannot be used by the relatively too small cytoplasma, which would lead to an enlargement of the nucleolus. Vice versa this fact explains the presence of inconspicuous nucleoli in case of little or no disturbance of the nucleolasmic ratio.

The appearance of prominent nucleoli in case of lacking mucous formation resp. their absence in case of existing mucous formation is explained on the same basis. The formation of mucus points to a yet existing differentiation of the cells and supposes a considerable cytoplasmatic activity, at which ribosomal RNA of the nucleolus is consumed. This occurs only in a small mesure in cells which solely reproduce themselves and whose metabolic activity is represented predominantly by the DNP-synthesis, so that it may get to an accumulation in the nucleolus.

The meeting of prominent nucleoli and increased mitoses may be interpreted equally as a loss of differentiation: the secretion of proteins and so also the demand for ribosomal RNA remains restricted in tissues with a metabolic activity largely limited to the synthesis of DNP. The cells of disdifferentiated carcinomas apparently start with renewed DNP-synthesis immediately after the mitosis. Lipkin (1971) was able to show by marking with H^3-Thymidine that in human colon-carcinomas the duration of the S- and G2-phase is very much prolonged.

The observation that the tumors' mucous production depends upon the localization indicates, that in the majority of cases there still remains a certain differentiation; for that speaks the more frequent ocurrence of muciparous carcinomas in the colon than in the rectum. In this connexion it is to be considered that in the healthy intestinal tract the mucous formation of the epithelium (from which the neoplasma originates) also decreases towards the rectum.

The cluster-analytic calculations, represented in Fig. 4, should help to discuss the great variety of the mentioned single results in close accordance with the statistically acquired formal structure (the figures in parentheses refer to the numeration of the variables in Table 1 and Fig. 4–6). In Fig. 4 the variables nucleoplasmic ratio (7), nucleoli (5) and death age of the patient are of high similarity. The character death age (1) shows an only medium resemblance to the sex (2), whereas for that character a surprising connexion with the tumor fibrosis is found. The dependence of observed metastases (4) on the death age was already discussed and connected with external factors of selection of the investigated collective. Nucleoplasmic ratio (7), nucleoli (5), mitoses (6) and eosinophilia (11) constitute formally a circle, at which the eosinophilia shows the lowest relation to the nucleoplasmic ratio on the one side as well as to the mitoses on the other side. With the interdependence between nucleoplasmic ratio and mitoses, these 4 variables result in a half-full circle. — What does that mean ?

The entire investigations were realized exclusively on routine slides stained by haematoxylin and eosin. This staining is an useful and proved routine method, but not appropriate to a detailed report about cellular nucleus and cytoplasma.

Therefore, besides the substantial statement of the findings, it must be supposed that the technique of staining and cutting leads to a purely technique-depending "shifting". This supposition is important for the judgement of the given characteristics. The packed bunching of mitoses (6), infiltration of eosinophilic granulocytes (11), nucleoli (5) and nucleoplasmic ratio (7) results without doubt in a substantial interpretation of the whole, in a wide-mashed manner recorded event, but precisely the very narrow cluster of these variables (half full circle) indicates common conditions, which, apart from the to be recorded characteristics and facts, already might be properties of the variables themselves and so of the date. This should be illustrated by the registration of mitoses, which is of limited possibility in the H-E-slide: here it is not only nearby impossible to differentiate the phases of mitoses, but single phases (such as the prophase) cannot be visualized at all by those means. A not to be neglected part of the total mitoses might not be observed even knowing the mitotic ratio and their chronological course within the particular phases. This mechanically conditioned „failure" may play an even greater part in the recording of eosinophilia (11) (and so also of the nucleoplasmic ratio -7-) and above all, although by different cause, in the recording of the nucleoli.

In Fig. 5 the "external" behaviour of the tumor (metastasizing yes or no — restriction of this statement by external selection, comp. above) is being opposed to features of the entire tissue (so called "inflammatory accompanying reaction" -10-) and to variables of attributes proper to cells. At that, for the variables 6–7–11 (Fig. 5) applies the same restriction as already discussed in connexion with Fig. 4. However, the chain: metastasizing (4) — lymphoplasmocytic infiltration (10) — eosinophilia (11), with the branching mitoses (6) and nucleoplasmic ratio (7), "explaines" surprisingly well the tumor's behaviour in the entire organism (metastasizing -4-) and the behaviour of the tumor tissue (lymphoplasmocytic infiltration -10-) by the cytological characteristics: mitoses (6), nucleoplasmic ratio (7) and eosinophilia (11).

A similar, somewhat modified cluster is reproduced in Fig. 6: the high similarity between the variables "localization" (3) and "mucous formation" (8) attracts attention in the first instance. These form a chain of outstanding similarity together with the characteristics "nucleoli" (5) and "mitoses" (6). Here the variables 6–7–11 (mitoses, nucleoplasmic ratio and eosinophilia) also attach to the variable 5 (nucleoli) as in Fig. 4 and 5. While the first two cluster-variables of cell and tissue characteristics bunch together and are related chiefly to the age of death (1), sex (2) and metastasizing (4), in Fig. 6 the formal coordination is determined above all by mucous formation and localization.

Each of these clusters describes one aspect of the entire tumor event. One might graphically visualize that the variables, as an intermediate stage of the observed facts, form a polydimensional room, whose projections on a bidimensional plane (as in Fig. 5) place certain coordinations into the foreground and neglect others or don't permit them to appear. The spatial "position" of this projection plane as well as the decision if a variable appears or not in a determined figure (cluster), is a function of the previously calculated similarity rate of the PHI-correlation-coefficient (Table 1, upper half). Precisely these methodics — how strange it might seem at first — could rather correspond to the urge of interpretation of an investigator than the merely rigid and "faceted" statement of "signifi-

cant" of "not significant" dependent conditions. By any means the morphologist sees more than only the "checked off" character, he also sees more than what corresponds to a great number of variables. What he recognizes as a rule not easily is the interdependence of single variables in a complex general event. However in this case the conventional statistics could help him on inasmuch as they rendered connexions between different variables "possible". Only the multifarious statistics could offer the synopsis of variables formally brought together and judged on the (recorded) whole — in our view a decisive urge of the morphologist. Above all they have to be considered as a methodical "coupling link" between the investigator in person and the facts observed as characteristics with the applied scales in the formal intermediate stage (in the hereby discussed examples the scales consist only of two values: "present" or "yes" and "not present" or "no"). At that the discussion and interpretation of the characteristics certainly is in the foreground, but the methodical conclusion of this standard circle till the conception of the model of investigation is at least equally significant. As shown in Fig. 4, the significance of the methods of investigation and processing as well as of the employed measuring instruments (scales of variables) increases with the complexity of the whole recorded characteristics (the number of variables investigated at the same time may serve as an indication of that fact). So, analyzing Fig. 4 for instance, there are found connexions between variables, whose interpretation exposes entirely different facts:

1 External factors of selection: metastasizing and age of death (1–4);

2 methodic-technical inadequacies: nucleoplasmic ratio, nucleoli, mitoses, eosinophilia (7–5–6–11, half-full circle);

3 dependences which characterize facts of the tumor biology: e.g. nucleoli and mucous formation (5–8);

4 connexions of unknown nature which are at the moment beyond interpretation: sex and tumor fibrosis (2–9).

For that, we recommend for each investigation (also the morphological) a method of evaluation which should be "sensitive" enough to reveal also the conditions of the investigation and recording of the characteristics.

References

Bacon, H. E.: Cancer of the colon, rectum and anal canal. Philadelphia: J. B. Lippincott Comp. 1964

Bagshaw, K. D.: Immunological features of choriocarcinoma. Brit. med. J. 1970 IV, 426–427

Black, M. M.: Reactivity of the lymphoreticuloendothelial system in human cancer. In: Progress in clinical cancer, vol. I, ed. I. M. Ariel. New York: Grune & Stratton 1965

Black, M. M., Opler, S. R., Speer, F. D.: Microscopic structure of gastric carcinomas and their regional lymphnodes in relation to survival. Surg. Gynec. Obstet. **98**, 725–734 (1954)

Bokelmann, D., Drüner, H. U., Schulz, U.: Klinik und Prognose der Kolon-Rektum-Karzinome. Dtsch. med. Wschr. **97**, 1590–1594 (1972)

Broders, A. C.: Squamous cell carcinoma of the lip; a study of 537 cases. J. Amer. med. Ass. **74**, 565–664 (1920)

Broders, A. C.: Carcinoma; Grading and practical application. Arch. Path. **2**, 376–381 (1926)

Brunschwig, A., Southam, C. M., Levin, A. G.: Host resistance to cancer: Clinical experiments by homotransplants, autotransplants and admixture of autologous leucocytes. Ann. Surg. **162**, 416–423 (1965)

Burkett, L. L., Field, M. L., Diggs, L. W.: A possible immune reaction producing spontaneous remission in leukemia. Blood **25**, 541–547 (1965)
Burkitt, D. P.: Epidemiology of cancer of colon and rectum. Cancer (Philad.) **28**, 3–13 (1971)
Crowther, D., Fairley, G. H., Sewell, G. L.: Significance of the changes in the circulating lymphoid cells in Hodgkin's desease. Brit. med. J. 1969 II, 473–477
Diamandopoulos, G.T.: Plasma cell and lymphocytic reaction in the syrian hamster to transplanted homologous tumor cells transformed in vitro "spontaneously" or by SV40. Arch. Ges. Virusforsch. **22**, 108–121 (1967)
Dukes, C. E. (ed.): Cancer of the rectum. Edinburgh: Livingstone 1960
Fabrikant, J. I.: The kinetiks of cellular proliferation in human tissues. Determination of duration of DNA-synthesis using double labeling autoradiography. Brit. J. Cancer **24**, 122–126 (1970)
Freeman, M. F., Tukey, J. W.: Transformations related to the angular and to the square root. Ann. Math. Statist. **21**, 607–611 (1950)
Hannig, K., Warba, H.: Isolierung von vitalen Tumorzellen durch trägerfreie Elektrophorese. Z. Naturforsch. **19b**, 860 (1964)
Haseloff, O. W., Hoffman, H.-J.: Kleines Lehrbuch der Statistik. Berlin: Walter de Gruyter & Co 1970
Higginson, J.: Etiological factors in gastrointestinal cancer in men. J. nat. Cancer Inst. **37**, 527–545 (1966)
Hoffmann, J., Post, J.: In vivo studies of DNA synthesis in human normal and tumor cells. Cancer Res. **27**, 889–902 (1967)
Hoepke, H.: Wehrt sich der Körper gegen Geschwülste? Strahlentherapie **93**, 196–212 (1954)
Höpker, W.-W.: Informatik in der Pathologie. Statistische Grundlagen methodischer Betrachtungsweisen. Boehringer Mannheim GmbH (1970)
Humphrey, J. H., White, R. G.: Immunologie, Ed. 2, 235. S. Stuttgart: Georg Thieme 1972
Ivanyi, D.: Number and size of nucleolo in binucleated liver cells. J. Cell Biol. **51**, 313–317 (1971)
Jong, de: The distribution of cancer within the large bowel. Unveröffentl. Manuskript (1972)
Lieb, L. M.: In vitro uptake of tritiated thymidine by carcinoma of the human colon. Cancer Res. **26**, 733–740 (1966)
Linder, F.: Colon- und Rectumcarcinom. Verh. dtsch. Ges. Chir. **88**, 302–311 (1971)
Lindholm, L., Rydberg, L.: Development of plasma cells during acute graft-versus-host reactions. Acta path. microbiol. scand. **B79**, 445 (1971)
Lipkin, M., Bell, B., Stalder, G., Troncale, F.: The development of growth abnormalities in colonic epithelial cells of man, W. Burdette, ed. Springfield/Jll.: Ch.C. Thomas, 1970
Love, R., Walsh, R. J.: Nucleolinar morphology in normal diploid, neoplastic, and aneuploid cells in vitro. Cancer Res. **30**, 990–997 (1970)
Maden, B. E. H.: Ribosoma formation in animal cells. Nature (Lond.) **219**, 685–689 (1968)
Mayr, E.: Zur Prognose des Kollumkarzinoms. Strahlentherapie **92**, 67–74 (1953)
McCarty, W. C.: Factors which influence longevity in cancer; a study of 293 cases. Ann. Surg. **76**, 9–12 (1922)
McQuitty, L. L.: Elementary linkage analysis for isolating orthogonal and oblique types and typal relevances. Educ. Psych. Measmt. **17**, 207–229 (1957)
Nagel, G. A., Geiger, Ch.: Krebs und Immunität. Schweiz. med. Wschr. **101**, 1605–1620 (1971)
Old, L. J., Boyse, E. A.: Immunology of experimental tunors. Ann. Rev. Med. **15**, 167–186 (1964)
Perry, R. P.: The nucleolus and the synthesis of ribosomes. Progr. Nucl. Acid Res. **6**, 220 (1967)
Ruhenstroth-Bauer, G., Rueff, F., Fuhrmann, G. F.: Die Zellelektrophorese in der klinischen Diagnostik. Elektrophoretische Untersuchungen an Blut-, Lymphknoten- und Tumorzellen von frisch gewonnenen Proben. Münch. med. Wschr. **105**, 1242–1250 (1963)
Sachs, L.: Statistische Auswertungsmethoden. Berlin-Heidelberg-New York: Springer 1968
Sandritter, W., Schiemer, H. G., Alt, W.: Das Interferenzmikroskop im Dienste der Cytologie und Krebsforschung. Klin. Wschr. **38**, 590–595 (1960)
Schoch, G.: Über die lokale Eosinophilie bei Karzinom. Zbl. Gynäk. **50**, 2895–2900 (1926)
Shepherd, J. M., Jones, J. S. P.: Adenocarcinoma of the large bowel. Brit. J. Cancer **15**, 680 (1971)

Sherwin, R. P., Zovickian, A.: Squamous carcinoma of the oral cavity and throat. Its clinicopathologic correlation. Amer. J. Surg. **102**, 823–829 (1961)
Southam, C. M., Brunschwig, A., Levin, A. G., Dizon, Q.: Effect of leucocytes on transplantability of human cancer. Cancer (Philad.) **19**, 1743–1753 (1966)
Steel, G. G., Bensted, J. P. M.: In vitro studies of cell proliferation in tumors. I. Critical appraise of methods and theoretical considerations. Europ. J. Cancer **1**, 275–279 (1965)
Steffen, C.: Allgemeine und experimentelle Immunologie und Immunpathologie, S. 80. Stuttgart: Georg Thieme 1968
Stewart, T. H. M.: The presence of delayed hyposensitivity reactions in patients toward cellular extracts of their malignant tumors. 2. A correlation between the histoloic picture of lymphocyte infiltration of the tumor stroma, the presence of such a reaction and a discussion of the significance of this phenomenon. Cancer (Philad.) **23**, 1380–1387 (1969)
Studzinski, G. P., Gierthy, J. F.: Cytologic appearance of the nucleolus of normal and neoplastic cells in relation to the synthesis of RNA. Act. cytol. (Philad.) **16**, 245–248 (1972)
Tschahargane, C., Haag, D., Goerttler, Kl.: Über den Altersformwandel der Zellkerne in der menschlichen Epidermis. Z. Zellforsch. **119**, 434–450 (1971)
Woodruff, M. F. A.: Immunological aspects of cancer. Lancet 1964 II, 265–269
Wynder, E. L., Shigamatsu, T.: Environmental factors of cancer of the colon and rectum. Cancer (Philad.) **20**, 1520–1561 (1967)

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