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ROLE OF INFLAMMATION IN CARCINOGENESIS*

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The relation of inflammation to cancer is a problem that has interested investigators for many years. Broussais in 1826 seems to have been the first to state that cancer is determined by chronic inflammation. For example, he expressed the view that gastric cancer was preceded by a chronic gastritis. To Broussais chronic irritation was the important factor in the pathogenesis of cancer. With the advent of the cellular theory in pathology, the earlier views of Broussais were either forgotten or neglected. Virchow in the middle of the nineteenth century interpreted the presence of leucocytes around a neoplasm as evidence that a malignant tumour was developing. Virchow (1871) attributed tumour formation to chronic irritation. In modern times these early opinions have been discarded by some, while others have adhered to them.

Ewing (1940) has made an excellent survey of the problem. Various forms of chronic inflammation, according to Ewing, have been observed to precede the appearance of numerous tumours. Precancerous conditions represent in many instances various forms of chronic inflammation. These may be manifested in several different forms such, for instance, as cicatrices from burns or syphilitic lesions. Wolbach (1925) studied the effects of repeated x-ray exposures upon the skin antecedent to the formation of carcinoma. He pointed out that the reactions of the epidermis are probably secondary to lesions in the corium. For many years after x-ray injury the epidermis proliferates and behaves as in any other reparative process. But, according to Wolbach, continuous proliferative activity in an abnormal supporting tissue predisposes to the development of malignant properties. After viewing many of the forms of precancerous inflammatory conditions, Ewing (1940, 527) arrives, for instance, at these conclusions: "In routine material I find transition stages from hypertrophic endometritis to carcinoma rather frequently, but clinical observations are still inadequate to prove the close dependence of the malignant upon the inflammatory process." In regard to the transformation of benign into malignant tumours, Ewing concludes that some benign growths appear to originate from inflammatory processes and that the involved tissues may present all gradations between single proliferative activity, adenoma, and carcinoma.

Laurens (1941) has pointed out that since carcinogenesis by ultra-violet irradiation is usually preceded by severe erythema, it is possible that the production of neoplasia is related to chronic inflammation. In general,

there has been considerable study and evidence on the relation of irradiation to chromosomal aberration and subsequent neoplastic formation. Lacassagne (1933) infected rabbits with a micro-organism, *Streptobacillus caviae*. The infection subsided under the influence of irradiation. Six months to four years later, tumours were found to have developed at the site of irradiation. From these observations Lacassagne concluded that local inflammation seems to be an important factor in carcinogenesis. Burrows and his collaborators (1937) have essentially confirmed these observations. They have induced foci of inflammation in the groin of rabbits by injecting kaolin or silica suspended in olive oil. These areas were subsequently irradiated. Within two years tumours were found to have developed in six out of nine animals.

A number of years ago studies were initiated in my laboratory in an endeavour to throw further light on the role of inflammation in carcinogenesis. I propose to bring to your attention the results of these various studies.

Mechanism of Repair and Formation of Precancerous-like Lesions

The studies of the past twenty-five years have indicated that injured cells in an inflamed area liberate various chemical mediators, which can satisfactorily explain some of the biological manifestations of inflammation (Menkin, 1956a, 1956b). A search was undertaken to determine whether a similar mediator may not likewise be present in exudate which in turn can reasonably explain the mechanism of repair in inflammation. One has to recall that the exudate contains to a large extent the products of cell injury admixed, to be certain, with elements from the circulating blood. The use of blood serum can adequately control for the non-cellular products seeping from the blood-stream. When exudative material obtained from an inflamed pleural cavity of a rabbit is repeatedly injected for months into the tragal part of the ear of rabbits, there developed, several months after cessation of the course of injections, local nodular prominences (Menkin, 1941). Microscopical cross-section through the normal ear reveals a linear-appearing cartilaginous plate and the usual superficial epithelial layer. On the other hand, a section through the area in which repeated injections of exudative materials had been performed reveals a heterogeneous cartilaginous trabecular pattern, or the proliferation of newly formed cartilaginous cells arising from the perichondrial layer (Fig. 1). Occasionally even metaplastic bone transformation may be encountered. The epithelial layer displays considerable degrees of

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hyperplasia with keratin formation, and "tongues" in the epithelial layer dipping down into the corium (Fig. 2) (Menkin, 1941).

When numerous injections of serum, as control, are introduced into the tragal region of the ear, even seven-and-a-half months after cessation of the course of injections, there is no change in the appearance of the cartilaginous plate (Menkin, 1941). When croton oil emulsified in rabbit serum is repeatedly introduced into the tragus of the ear of rabbits for several months, there

FIG. 1.—Section through the ear of a rabbit. This animal had received 20 injections of exudative material in the ear during a period of about four months. Seven and a half months after cessation of the injections, biopsy revealed marked proliferative activity in the perichondrial region. ($\times 60$.) (Taken from Menkin, 1941.)

FIG. 2.—Drawing of microscopical section through the ear of a rabbit at the site of seven successive injections of exudate. Note the hyperplasia of the epithelium dipping downward into the corium and the foci of keratin formation.

follows an acute inflammation, which over a period of seven months after the cessation of the course of injections ultimately subsides. Examination of the cartilaginous plate reveals no evidence of any proliferative response. The effect of the injections of exudative material is therefore not directly referable to the inflammation or the repeated injury induced by an irritant *per se*. The observations rather suggest the presence of a growth-promoting component released by injured cells into the exudate. Its presence may give a reasonable explanation for the mechanism of repair after the acute inflammatory process.

An attempt has been undertaken to separate this proliferative factor from the exudate. Fairly satisfactory results have finally been obtained by a very simple procedure (Menkin, 1953, 1956b, 1957). The exudate, usually of canine origin, is dialysed against distilled water for several hours by constantly agitating the "cellophane" tube. The diffusate is then concentrated to about one-tenth its original volume. Intracutaneous injection of this material scarcely induces any inflammatory response, in contradistinction to the toxic euglobulin necrosin, extracted from the indiffusible material remaining in the cellophane tube. Even repeated cutaneous injections of the concentrated diffusate yield a considerably reduced type of effect when compared with that obtained with the necrosin recovered in the indiffusible material. Repeated injections of the concentrated diffusate into the tragus of the ear tends to produce either a mild degree of firmness or else tiny nodular prominences. Microscopical studies may reveal in some parts of the ear newly formed cartilaginous cells growing from the perichondrial layer. On the other hand, a diffusate prepared from canine blood serum, for instance, even after 19 injections during two months, induces no visible change in either the epithelial layer or the cartilaginous plate.

A search has been undertaken for a site that is more responsive to the injections of the diffusate than the tragal region of the ear. Such a region has been found in the breast tissue of non-pregnant rabbits (Menkin, 1953, 1956b, 1957). Repeated injections of concentrated diffusate material in the vicinity of the nipple induce hyperplasia of breast tissue. Even after two weeks, with as few as 11 injections, the hyperplastic involvement of acini and ducts may become quite striking. An untreated nipple in the animal fails to yield any hyperplastic response on the part of the lining epithelium. Concentrated blood-serum diffusate after 12 injections in a period of three weeks into breast tissue of non-pregnant rabbits yields nothing remarkable. On the other hand, 12 injections of concentrated diffusate of exudate in the same period and in the same rabbit induce cystic dilatation of the ducts. When the period of injections is extended, there may be found considerable connective-tissue stroma, and the picture may even be reminiscent of an intracanalicular fibroadenoma. The ducts may at times appear dilated with hyperplasia and even squamous metaplasia of their lining epithelium. The picture recalls that encountered in the chronic cystic mastitis of human beings.

The active principle in the concentrated diffusate has been found to be readily precipitated with acetone (Menkin, 1956b). This precipitate, washed with distilled water and suspended in saline, likewise shows marked hyperplastic responsiveness. Cystic dilatation of the ducts and epithelial "pearl" formation is a frequent

outcome of repeated injections of the acetone-precipitate of the concentrated diffusate (Fig. 3). Dilated ducts may reveal squamous metaplasia and pseudostratification, besides reduplication of the epithelium. As stated above, the picture recalls that of a chronic cystic mastitis. To repeat, neither the diffusate of blood serum nor saline injections reproduce any such effects; and the resulting picture is not appreciably greater than that of an untreated nipple in the same rabbit.

The growth factor of exudates affects likewise the cutaneous epithelium and hair follicles in the vicinity of the injected nipples. Nature's contribution of several nipples renders this a propitious animal. One of the nipples, for instance, can be utilized as an untreated control. The hair follicles in its vicinity show nothing remarkable. After numerous injections of the concentrated diffusate—for example, 85 injections—for six months—marked hyperplasia of the cutaneous follicular epithelium is seen. The acetone-diffusate precipitate likewise causes a marked hyperplastic response (Fig. 4). As control, the repeated injections of saline induce no effect on the follicular epithelium.

A study was undertaken to determine whether the combination of the growth factor of exudate and a carcinogenic hydrocarbon would induce a synergistic or enhanced effect (Menkin, 1959). Rous and Friedewald (1944) reported that the application of methylcholanthrene and tar to virus-induced papillomas of the domestic rabbit hastens the formation of carcinomatous structures, and that the malignant changes are often multiple. Rogers and Rous (1951) also showed that scarified areas of rabbit skin previously rendered hyperplastic with turpentine and then inoculated with Shope papilloma virus develop carcinomatous changes in many cases, after several months, provided that the areas were also covered with a dressing that contained 20-methylcholanthrene or 9:10-dimethyl-1:2-benzanthracene. According to these investigators the virus and the hydrocarbon seemed to act jointly in their carcinogenic capacities.

Deelman (1924) had reported that wounds in a tarred region hasten and localize the formation of tumours. Orr (1935) reported that fibrosis of the subcutaneous tissue caused by the insertion and subsequent removal of linen threads induces accelerated tumour in mice by means of 1:2:5:6-dibenzanthracene. Cramer (1929) found that injury by cautery to a tar-wart papilloma tends to favour the development of malignancy. According to Berenblum (1941, 1944), once a preneoplastic state has been induced by a true carcinogen a

FIG. 3.—Twenty injections of the acetone precipitate of the diffusate of an exudate for about one month. Note the epithelial "pearl" formation and the epithelial hyperplasia around part of a widely dilated duct. The epithelial layer in the duct displays a pattern of pseudostratification with squamous metaplasia. The picture is somewhat reminiscent of the chronic cystic mastitis seen in human beings. ($\times 111$.) (Taken from Menkin, 1956b.)

FIG. 4.—Appearance of hair follicles six and a half months after 85 injections in the nipple of the acetone precipitate of the diffusate of exudates suspended in saline. The hyperplasia and keratosis are striking but there are no signs of any invasiveness. ($\times 108$.)

TABLE I.—*The Effect of the Growth-promoting Factor of Exudates with or without Superimposed Paintings with Methylcholanthrene on the Breast Tissue of Rabbits*

Rabbit No.	No. of Injections of Growth-promoting Factor and Number of Paintings with Carcinogenic Hydrocarbon	Duration of Experiment (Months)	Volume of Breast Tissue Underlying Nipple (c.cm.)				
			Untreated Nipple	Nipple Painted with Methylcholanthrene	Nipple Injected with Concentrated Diffusate	Nipple Injected with Concentrated Diffusate Plus Painting with Methylcholanthrene	Nipple with Methylcholanthrene Plus Saline Injections
28-89	54	4.5	0.36	1.42	{ 0.83 4.88	{ 6.3 17.21	
38-93	60	6	0.75	0.64	{ 1.41 1.57	9.61	
28-97	127	11.5	0.22	0.49	1.14	{ 4.13 5.14	0.56
28-58	127	11.5	0.45	0.27	2.08	{ 11.07 6.18	1.09
Average	92	8.4	0.45	0.71	1.99	8.52	0.83

FIG. 5.—Rabbit 29-27. 88 injections of concentrated diffusate in the vicinity of a nipple followed by 88 paintings of 1% methylcholanthrene in acetone for eight months has induced marked proliferative introductal activity of the lining epithelium with squamous metaplasia. ($\times 139$.)

benign tumour can be made to appear at that site. Subsequent progress to a carcinoma can then be hastened by a variety of non-carcinogenic irritants. Boutwell, Bosch, and Rusch (1957) have demonstrated that small amounts of croton oil applied to susceptible mice induce papillomas and, subsequently, carcinomas in a certain percentage of treated animals. The response to croton oil is greater when the mice have previously been treated with a subcarcinogenic dose of 9:10-dimethyl-1:2-benzanthracene.

The observations on the combination of the growth factor and methylcholanthrene indicate a synergistic effect. In Table I are assembled some typical results of several experiments. The volume of breast tissue underlying each nipple is obtained by measuring length, breadth, and thickness with a calliper. Typical measurements collected in Table I indicate that the growth factor induces an average increase in volume amounting to almost five times that of the untreated nipples; whereas the combination of the growth factor and a carcinogenic hydrocarbon results in an average increase in volume of almost 20 times that of the controls (Table I).

Methylcholanthrene paintings alone fail to induce any significant increase in volume of breast tissue. After 88 paintings of 1% methylcholanthrene in acetone for a period of eight months, one encounters no appreciable change on the part of the lining epithelium in ducts and acini; while the repeated injections of the growth factor followed by paintings with the carcinogenic hydrocarbon for a similar period induce marked epithelial growth into the lumina of some of the ducts and acini (Fig. 5). Prolonged administration, such as 54 injections of the diffusible growth factor followed by paintings with methylcholanthrene for six months, induce in rabbits the formation of papillomas. This is not encountered when there are only repeated injections of the concentrated diffusate, and infrequently by merely repeated paintings. It therefore appears as if

FIG. 6.—Rabbit 44-22. 52 injections of concentrated diffusate of exudate in the nipple region followed by 52 paintings of 1% methylcholanthrene in acetone over a period of four and three-quarter months has induced in the treated cutaneous area a squamous carcinoma. ($\times 123$.)

the growth factor of exudates behaves as a cocarcinogen-like substance to a carcinogenic hydrocarbon—that is, methylcholanthrene. When the concentrated diffusate of blood serum is substituted for that of exudate, no such accelerated development of papillomas tends to occur in a similar interval.

In conclusion, it appears as if the role of inflammation in carcinogenesis is that of a cocarcinogen via the growth factor of exudates. It is difficult to induce a cancer in rabbits with a carcinogenic hydrocarbon. The combination of the growth factor of exudates and methylcholanthrene has in very few cases induced a

FIG. 7.—Rabbit 44-22. The squamous carcinoma illustrated in Fig. 6 has invaded the corium. Note "pearl" formation in one of the carcinomatous islets. ($\times 200$.)

squamous carcinoma. This is illustrated in Fig. 6. This effect has been obtained after 52 injections of the growth factor and 52 local paintings of the carcinogenic hydrocarbon for a period of four and three-quarter months. Painting alone for the same interval has merely induced hyperkeratosis of the hair follicles and hyperplasia of the epithelium. The squamous carcinoma induced by the combination of the growth factor and the hydrocarbon invades the deeper layer of the corium (Fig. 7). In brief, it is conceivable that the studies of numerous investigators, some of whom have been cited above, on the mechanism of cocarcinogenicity by various irritants—for example, croton oil—may be referable to the induction of an inflammation. Following the eventual mild degree of cellular injury, and the consequent release of the growth-promoting factor, there may be a hastening in development of a neoplasm in the presence of a carcinogen.

Irreversible Effect Induced by the Growth-promoting Factor of Exudates

When the growth factor is injected for a relatively brief period, the enlarged nipple tends to regress. When, however, the course of injections is maintained for several months and is at least above 30, there is a tendency for a state of irreversibility to be established even though administration of the active growth material is discontinued. This is illustrated in Figs. 8 and 9. In this experiment the breast was injected 61 times for a period of five and a half months. The injections were then discontinued. Three months later

FIG. 8.—Rabbit 27-07. For almost five months the nipples of this non-pregnant rabbit were treated as follows. A: untreated. B: 61 injections of the concentrated diffusate of an exudate. C: 61 injections of physiological saline. D: 61 paintings with 1% methylcholanthrene in acetone (the first 22 paintings consisted of methylcholanthrene in olive oil 5% and 1%). E and F: 61 injections of the concentrated diffusate of an exudate plus 61 paintings with 1% methylcholanthrene in acetone (the first 22 paintings consisted of methylcholanthrene in olive oil 5% and 1%). At this stage the B and E nipples show definite enlargement. The administration of the concentrated diffusate or the paintings, or of both, were discontinued, and the nipples were subsequently studied for any irreversible effects (see Fig. 9). FIG. 9.—Rabbit 27-07. About three months after cessation of injections of the concentrated diffusate of exudate in the B area the enlargement of the nipple has not regressed. In fact, it appears to be somewhat more prominent than at the termination of the course of injections (see Fig. 8). Note, therefore, the irreversible effects induced by the repeated injections of the proliferative factor of exudates. A similar irreversibility of effect seems to have occurred in the E nipple, in which repeated concentrated diffusate injections and paintings had been administered prior to cessation three months before.

B

FIG. 10.—The concentrated diffusate of an alkaline exudate is at an alkaline pH. The ultra-violet absorption curve levels off at 250 mμ, and this is maintained until about 285 mμ.

not only had the volume of the breasts not regressed, but, if anything, they had enlarged further. Nevertheless, the growth factor *per se* can only favour, after multiple injections, the establishment of a relative irreversible state; yet by itself it fails to induce a neoplasm. Rather, as mentioned already, it behaves as a cocarcinogen, thus pointing to a definite role of inflammation in carcinogenesis via the growth-promoting factor of exudates.

On the Nature of the Growth-promoting Factor of Exudates

Studies have been undertaken in an endeavour to determine the nature of the growth factor in exudates. Chromatographic studies by my former associate, Dr. Kalnins, on the hydrolysate of the exudate indicated the presence of a peptide (Menkin, 1957). The amino-acids identified were leucine, valine, lysine, α -alanine, glycine, aspartic acid, and serine (Menkin, 1957). Glutathione seemed also to be present.

Further studies were carried out with a DU spectrophotometer on the concentrated diffusate of exudate. As the inflammatory reaction progresses, the exudate is at first at an alkaline pH level, eventually assuming an acid level (Menkin, 1934, 1940; Menkin and Warner, 1937). The pH of the concentrated diffusate parallels the changes in the hydrogen-ion concentration of the exudate. With the latter at an alkaline pH the concentrated diffusate is at about pH 8 or 8.5. The

ultra-violet absorption curve flattens out at 250 $m\mu$ and remains so until about 285 $m\mu$, when it dips down (Fig. 10). From an acid exudate, the concentrated diffusate assumes likewise an acid reaction. Ultra-violet absorption shows under such circumstances a peak at 260 $m\mu$ (Fig. 11).

The diffusibility, thermostability, and absorption in the nucleic acid range support the view that one is dealing with a nucleotide. Nevertheless, the flattening of the curve after 250 $m\mu$ in an alkaline diffusate was puzzling until it was found that adjustment of the pH to 4.5 or thereabouts with N acetic acid favours the formation of a small precipitate which augments in quantity under refrigeration. This precipitate can be collected by centrifuging it at 21,600 $\times g$. The ultra-violet absorption of the aqueous suspension yields a peak at 260 to 270 $m\mu$ (Fig. 12). Evaporation to dryness of a diffusate yields on suspension in an aqueous medium a precipitate with a peak in the peptide range. This fact,

FIG. 11.—The concentrated diffusate of an acid exudate is likewise acid. There is a peak of absorption at 260 $m\mu$ consistent with a nucleotide.

FIG. 12.—An alkaline diffusate derived from an alkaline exudate when adjusted with N acetic acid to pH 4–4.5, or thereabouts, forms a small precipitate. This precipitate suspended in an aqueous medium yields a peak of absorption at 265 $m\mu$, consistent with nucleotides.

in addition to the chromatographic studies by Kalnins on the acid hydrolysate of the active diffusate, as well as the inability of nucleotides to be precipitated at pH 4.5 by acetic acid, supports the view that the factor may well be a *nucleopeptide*. Descending chromatographic studies with three different solvents support the presence of a nucleotide or related groups in the growth factor (Table II). Finally, recent observations indicate that

TABLE II.—Chromatographic Studies on Growth-promoting Factor of Exudates

Type of Solvents Utilized	No. of Observations	Purine, Pyrimidine, Nucleoside, Nucleotides Identified	Frequency of Recovery by Ultra-violet Light
N-Butanol, ethanol, 5 N HCl (3:2:2)	6	Uridylic acid, uridine Cytidylic acid, cytidine Adenine Guanine	6 5 4 6
Aqueous N-butanol (86% by volume)	5	Uracil Cytosine, cytidine Adenine, adenine DR* Guanine Thymine, thymine DR* 5-Methylcytosine Hypoxanthine	4 4 5 2 3 2 1
Isopropyl alcohol 170 ml., conc. HCl 41 ml., H ₂ O 39 ml.	4	Uracil Cytosine, cytidine, cytosine DR* Adenine, adenosine Guanine, guanosine Thymine, thymine DR* Hypoxanthine Xanthine 5-Methylcytosine	2 4 2 2 3 3 1 2

* DR refers to deoxyriboside (Chargaff and Davidson, 1955).

TABLE III.—Effect of Ribonuclease on the Inactivation of the Growth-promoting Factor of Exudates

Rabbit No.	No. of Injections of Growth-promoting Factor With and Without Exposure to Ribonuclease	Duration of Experiments (Months)	Volume of Breast Tissue Underlying Nipple (c.cm.)			
			Untreated Nipple	Nipple Injected with Concentrated Diffusate	Nipple Injected with Concentrated Diffusate but Hydrolysed Previously with Ribonuclease	Nipple Injected with Distilled Water
5291	24	2	0.99	1.45 3.46	1.14 0.89	0.87
5292	28	2.5	0.58	2.24 3.02	0.16 1.90	0.39
5293	25	2.5	0.23	0.55 1.71	0.12 0.10	0.23
3303	15	1	0.91	1.53 0.96	0.48 0.81	—
Average	23	2	0.68	1.87	0.70	0.50

when the concentrated diffusate of exudates containing the growth factor is treated with trypsin or with ribonuclease, the active principle in the growth factor is inactivated (Figs. 13 and 14). The data in Table III indicate that ribonuclease inactivates the factor. This type of evidence with appropriate enzymes would perhaps support the characterization of the growth factor of exudates as a *nucleopeptide*. Further chemical studies, however, are in progress to substantiate this view.

This nucleopeptide is biologically active in inducing an enlargement of breast tissue. For instance, seven weeks after the cessation of a course of 34 injections of the nucleopeptide, the enlarged breasts are found conspicuously enlarged. Three and a half months after the termination of the course of injections, the breasts have not regressed in size (Fig. 15). Even six months afterwards the irreversible state of enlargement is to a great extent still present. The microscopical picture of the breast tissue seven weeks after 22 injections of the nucleopeptide displays a picture not unlike that seen in chronic cystic mastitis. There is marked proliferative activity of the epithelium into the lumen of the ducts (Fig. 16). Contrast this with the ducts in an untreated nipple in the same animal (Fig. 17). The cutaneous epithelium in the vicinity of the nipple is likewise hyperplastic after injections of the nucleopeptide. The hyperplasia is marked around the hair follicles. Follicular keratosis forms also a conspicuous feature.

In conclusion, the mechanism of repair in inflammation seems to be referable to liberation by injured cells of a diffusible growth factor, possibly a nucleopeptide, which can be recovered in exudates. Its repeated injections may induce a relatively irreversible state. In the breast tissue the resulting picture resembles that of a chronic cystic mastitis. The surrounding cutaneous and follicular epithelium becomes very hyperplastic after repeated injections of this active growth factor. Finally, in conjunction with a carcinogenic hydrocarbon the growth factor behaves as a carcinogen. The presence of such a tool liberated by injured cells defines the role of inflammation in carcinogenesis. It would appear as if inflammation through the growth factor acts as a cocarcinogen to an exogenous carcinogen, and even possibly to an endogenous carcinogen liberated as a result of genetic predilection. In other words, inflammation hastens and perhaps intensifies the neoplastic process, in turn initiated as a consequence of a genetic susceptibility or mutation.

Carcinogenesis and Cellular Injury

If I may express my own view, it seems conceivable that derangement in the biology of the cell leading eventually to neoplastic formation may be referable to a disequilibrium of the cell brought about by a variety of means. This may be in the form of a long-standing inflammatory process or chronic irritation, virus infection, or hormonal imbalance. The result of such disequilibrium may be a relatively low-grade injury to the cell which in turn releases the growth-promoting or proliferative factor described in this discussion (Menkin, 1956b). This factor *per se* is apparently

FIG. 13.—Rabbit 52-94. Breast tissue after seven injections of concentrated diffusate of exudate within a period of three weeks. Note the distended and cystic appearance of the ducts. On the upper right-hand corner some of the hyperplastic cutaneous epithelium is in evidence. ($\times 100$.)

FIG. 14.—Rabbit 52-94C. Breast tissue after seven injections of concentrated diffusate of exudate within a period of three weeks. The concentrated diffusate was previously hydrolysed with ribonuclease. Note in contrast to the illustration in Fig. 13 that there is no distension of the breast tissue, nor any cutaneous epithelial hyperplasia (lower left-hand corner). This observation on the inactivation of the growth factor with ribonuclease would indicate that activity is closely associated with the nucleotide part of the molecule in the growth-promoting factor. ($\times 100$.)

insufficient to develop a true tumour, but, as emphasized already, in a genetically susceptible organism or in the presence of a carcinogen it may favour neoplastic development (Menkin, 1956b).

To us, inflammation and neoplastic formation are both manifestations of cellular injury. Inflammation can be regarded as a manifestation of severe cellular injury in vertebrates, whereas neoplastic growth may be the expression of relatively mild protracted cellular injury with the consequent release of the growth-promoting factor, but in a genetically susceptible organism.

Hypotheses are useful only if they can furnish soil for further experimentation and therefore lead to scientific progress. With this point of view that the degree of injury may perhaps be a significant factor to consider, the following simple experiments were conducted.

About 70 rabbits received a mild type of long-standing injury by merely piercing the ear lobe with staples and fixing a metal tag held on the external surface of the ear (Menkin, 1955, 1956b). The extruding staples were bent and twisted on the inner surface of the ear. The tags were thus maintained for several months, and in

FIG. 15.—*Rabbit 19*. Effect of repeated nucleopeptide fraction injections on the breast tissue of a non-pregnant rabbit. Upper nipples A and B are untreated. Lower nipples C and D have been injected 34 times each with the nucleopeptide suspension of exudates over a period of three months. Photograph taken 3½ months after cessation of injections. Note that the nipples are markedly enlarged and have not regressed even though the course of injections has been discontinued for 3½ months.

FIG. 16.—*Rabbit 38-91D*. Appearance of a duct in the breast tissue of a nipple injected 22 times with the nucleopeptide of exudates over a period of seven weeks. Note marked hyperplastic effect of the epithelium as it protrudes into the lumen. The appearance recalls that encountered in the chronic cystic mastitis of human beings. ($\times 224$.)

FIG. 17.—*Rabbit 38-91A*. Appearance of ducts in an untreated breast tissue taken from same animal as illustrated in Fig. 16. The epithelial lining of the ducts is normal, in contrast to the experimental appearance shown in Fig. 16. ($\times 120$.)

FIG. 18.—*Rabbit 63-36*. Small circumscribed neoplastic growth in area of ear pierced about four months previously with staples to hold throughout that period the ear tag on the outer ear. Note that with mild degree of sustained injury new growths develop in about 25% of the rabbits studied. (Taken from Menkin, 1956b.)

FIG. 19.—Rabbit 63-36. Microscopical section of tumour shown in the gross in Fig. 18. The presence of islets of cartilage cells in a disorganized fibromatous stroma would suggest a chondrofibroma. The tumour has developed within a period of four months. ($\times 22$.) (Taken from Menkin, 1956b.)

two cases for over two years. About one-quarter of the rabbits developed small nodules at the site where the staples were extruding on the inner surface of the ear lobe (Fig. 18). Microscopical studies of these nodules revealed cartilaginous islets in a disorganized connective-tissue stroma (Fig. 19). The lesion is a benign tumour which may be termed a chondrofibroma. Biopsy is followed by secondary growth at the site of the excised tumour. An appropriate question that may be raised is why only 25% of the cases have developed tumours. There may be several interpretations on this point. To us it seems conceivable that only 25% of the rabbits carried genetic predilection to tumour formation. With the concomitant release of the growth-promoting factor from the site of mild injury over a prolonged interval, it is quite conceivable that the development of a local neoplasm occurs only in susceptible rabbits. This is one interpretation, but it is readily admitted that perhaps other explanations may be advanced.

Another experimental observation may be pointed out in regard to the view that the degree of cellular injury may be significant enough for consideration. The injury factor which determines the pattern of injury in inflammation is a euglobulin termed necrosin (Menkin, 1943). Repeated injection of necrosin into the nipple region of a non-pregnant rabbit is followed by a marked necrotizing inflammation. The architecture of the original tissue at the core of the abscess is completely obliterated (Fig. 20). On the other hand, at the periphery of the site of necrosin injections, where the concentration of necrosin is diminished and therefore the degree of injury is reduced, not only are dilated cystic ducts seen, but their epithelial lining may display evidence of proliferation, squamous metaplasia, pseudostratification (Fig. 21).

FIG. 20.—Rabbit 31-63. The effect of repeated injections of necrosin, the toxic euglobulin of exudates, on the breast tissue. Within a period of six weeks 17 injections of that substance were introduced into or in the vicinity of a nipple. Note the acute necrotizing reaction, with disappearance to a large extent of the normal architecture of the breast tissue. ($\times 142$.) (Taken from Menkin, 1956b.)

I believe that at this point I should stop, for our understanding of the complex mechanism at play has reached its limit for the present. For instance, we are wholly at a loss regarding the exact nature of the determinants concerned in the degree of induced cellular injury. Is it referable to an enzymatic disturbance or to the concentration of the irritant? Does the quantitative liberation of necrosin by an injured cell enter into play? These are all problems for the future to uncover. I only hope that curiosity has been aroused in an endeavour to understand further the significance and the mechanism of action of the endogenous growth-promoting factor liberated by injured cells.

Summary

The history of this subject, dating from the early studies of Broussais in France and Virchow in Germany

FIG. 21.—Rabbit 31-63. After 17 injections of necrosin, the injury factor of exudates, in the nipple region there follows within six weeks, as seen in Fig. 20, complete destruction of the core of the involved tissue. However, at the periphery of such areas, where the necrotizing effect is not quite so acute, one may encounter epithelial hyperplasia of ducts with pseudostratification. The picture is not wholly dissimilar to that met with in chronic cystic mastitis. ($\times 154$.) (Taken from Menkin, 1956b.)

to the more recent studies of Ewing, Lacassagne, and Burrows, is briefly described. In recent years I have demonstrated the presence of a growth-promoting factor in inflammatory exudates. This factor offers a reasonable explanation for the mechanism of repair. Its growth properties can be elicited by repeated injections in either the ear or the breast tissue of rabbits. In the latter area a picture resembling that seen in chronic cystic mastitis of humans is frequently obtained as a result of repeated injections of the growth factor in non-pregnant rabbits. In the ear, both epithelial and cartilaginous structures are affected in the form of marked proliferation from the perichondrium as well as pronounced hyperplasia and keratinization on the part of the epithelium.

The growth-promoting factor of exudates is diffusible, and as such it can be obtained by dialysis and subsequent concentration of the diffusate. It is heat-stable. Repeated injections tend to produce a state of irreversibility, so that the enlarged breast fails to regress upon cessation of the course of administration. Nevertheless, the factor *per se* fails to induce an autonomous neoplastic process. It has, however, been demonstrated that the growth factor of exudates behaves as a cocarcinogen when combined with a carcinogenic hydrocarbon—for example, methylcholanthrene. This appears to be the role of inflammation in carcinogenesis—namely, that of a co-carcinogen via the growth-promoting factor, liberated in turn by injured cells at the site of inflammation.

Absorption as well as chromatographic studies support the view that the growth-promoting factor of exudates contains peptide and nucleotide structures involved in its activity. The possibility that the factor is a nucleopeptide is envisaged, and is therefore being considered. Inactivation of the factor by both trypsin and ribonuclease supports such a view.

The problem of carcinogenesis and cellular injury is discussed. It is pointed out that severe cellular injury leads to the development of inflammation, while mild injury tends to produce proliferative activity. Basically, neoplastic formation appears to depend on genetic susceptibility, which, in turn, can be intensified, accelerated, or reinforced by the liberation of the endogenous growth-promoting factor of exudates, acting thus as a cocarcinogen.

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RHEUMATOID NEUROPATHY*

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"Nobody is on my side, nobody takes part with me:
 I am cruelly used, nobody feels for my poor nerves."

—Mrs. Bennett in *Pride and Prejudice*.

A sufferer from rheumatoid arthritis has enough to contend with in attempting to live in rough harmony with his painful, stiff, weak, and swollen joints. The addition of peripheral neurological features may make life even more unbearable for him and may necessitate admission to hospital and a greater degree of disability and crippling in the future. This complication of rheumatoid disease, for so we consider it, was forcibly brought to our notice three years ago, when four out of eight in-patients presented with sensory signs in the legs; one had motor weakness also, being unable to dorsiflex one foot. The fact that half our in-patients with rheumatoid disease at that time had peripheral neuritis was a pure coincidence, for the complication is relatively uncommon, but it started us on our present investigation (Hart *et al.*, 1957).

We have now seen a total of 25 such cases in our own unit and a further 17 in the clinics of colleagues who have kindly allowed us to see their patients and their records. An increasing number of cases have been reported in the last three years in the world's literature, mostly from the United States of America, where steroid therapy has largely been blamed for its emergence as a clinical entity. In this country, however, we have seen several cases which have never received steroids, and a neuropathy in rheumatoid disease was reported as long ago as 1887 by Pitres and Vaillard, in France, and in this country in 1898 by Bannatyne, who, in a book on rheumatoid arthritis, recognized it as a systemic disease and stated, "in a certain number of cases peripheral neuritis has been noticed, but in so far as to negative any idea that this [neuritis] is the cause of the [rheumatoid] disease"; and again, "in the cases where the reflexes are absent it is almost certain that we have to do with a neuritis probably set up by the joint inflammation or by toxins." The late W. H. Allchin (1900) wrote: "Definite peripheral neuritis has been demonstrated in a few cases [of rheumatoid arthritis] though not in the nerves which actually supply the joints."

In the last 30 years increasing emphasis has been placed on the systemic manifestations of rheumatoid arthritis (Fingerman and Andrus, 1943; Bauer and Clark, 1948; Calkins and Bauer, 1955), and a pathological study by Sinclair and Cruickshank (1956) has emphasized the widespread nature of rheumatoid

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