

INJECTION SITE TUMOURS AND PRECEDING PATHOLOGICAL CHANGES IN RATS TREATED SUBCUTANEOUSLY WITH SURFACTANTS AND CARCINOGENS

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Summary.—The sequential histological changes and neoplastic response occurring in the subcutaneous tissue of rats after injection of surfactants or carcinogens were compared. Twice weekly subcutaneous injections of the surfactants Blue VRS and Light Green SF elicited a deranged connective tissue repair with continued proliferation of fibroblasts and extensive collagen desposition. In contrast, the carcinogens N-methyl-N-nitrosourea (MNU) and N-nitroquinoline-N-oxide (NQO) appeared to inhibit connective tissue repair and produce morphologically abnormal fibroblasts. The spectrum of neoplastic response was also found to differ. Surfactants gave rise to local sarcomata only after about 47 weeks, whereas carcinogens produced local sarcomata and adenocarcinomata after 20 and 12 weeks respectively.

WE have previously reported the results of investigations of the chemical, physical and biological factors involved in the production of local sarcomata in rodents by repeated subcutaneous injection of a variety of chemicals. The relevance of these sarcomata in terms of the potential carcinogenic risk of such chemicals to man have been evaluated (Grasso *et al.*, 1971; Hooson and Grasso, 1971). These investigations were based on a sequential study of the local tissue reactions to repeated injections and the analysis of the physical properties of compounds involved. A correlation was found between the early lesion induced in the subcutaneous tissues by a short-term series of 10–20 injections given at the same site and the production of local connective tissue tumours by a long-term series of injections. Histologically, it was found possible to distinguish 4 types of reaction (types I–IV). Type I and II reactions were mild and self-limiting and did not progress to neoplasia. In contrast, type III and IV reactions were progressive and proliferative and injection site sar-

comata were invariably produced subsequently (Grasso and Golberg, 1966a). It was found that compounds eliciting a type III or IV reaction possessed certain physicochemical properties (surface activity, hypertonicity, etc.) capable of producing cell injury. Thus it appears that sarcomata which followed the induction of a type III or IV reaction were an indirect result of repeated injury to local fibroblasts and not due to a process of direct chemical carcinogenesis.

In contrast, a short-term study of the lesions induced by repeated subcutaneous injection of water soluble carcinogens, whose oncogenic potencies have been demonstrated by other methods of administration, gave different results. The lesion was characterized in all cases by an inhibition of the normal reparative response and the appearance of cytologically abnormal cells (Hooson and Grasso, 1971). We felt that it was important at this stage to extend these observations from the initial 5 weeks and to compare (1) the changes occurring from this time up to the appearance of tumours (the intermediate period)

and (2) the spectrum of neoplastic response produced by carcinogens with that produced by non-carcinogenic but irritant compounds.

Accordingly, 2 surfactant food colourings, Light Green SF and Blue VRS, which gave type III and IV responses respectively on short-term test, were injected in aqueous solutions into rats twice weekly and the progress of the lesion examined at frequent intervals. Two water soluble carcinogens, *N*-methyl-*N*-nitrosourea (MNU) and *N*-nitroquinoline-*N*-oxide (NQO) were administered under the same experimental regimen and the tissue reaction during the intermediate period, and the overall neoplastic response, were compared. These observations were made over periods of time up to 60 weeks.

MATERIALS AND METHODS

Animals.—Details of animals used are given in Table I, together with the experimental regimens employed. On average rats weighed 100 g at the start of experiments. The animals were maintained at a temperature of $22^{\circ} \pm 1^{\circ}\text{C}$ and at 50% relative humidity; they had free access to Spillers Small Laboratory animal diet and water. All animals were inspected twice weekly and the injection site shaved with electric clippers.

Chemicals.—Light Green SF sodium salt and Blue VRS sodium salt were obtained from the Food Colours Committee of the Chemical Industries Association. The specifications of these colourings were given in previous publications (Grasso and Golberg, 1966*b*). MNU was obtained from K and K Laboratories Incorporated, Plainview, N.Y. NQO was supplied by the Daichi Pure Chemical Co. Ltd., Tokyo, Japan.

Solutions.—Solutions of all chemicals were prepared using CO_2 free distilled water. Colourings were filtered and buffered to pH 7.0 before injection. Aqueous solutions of NQO and MNU at the concentrations used in these experiments had a pH of 7.2 and 6.7 respectively. All solutions were freshly prepared before injection.

Details of concentrations, volumes and frequency of injection are given in Table I.

All injections were given as far as possible into exactly the same site, the right flank.

Conduct of experiments.—The numbers of animals used and details of treatment are shown in Table I. Equal numbers of male and female animals were used in each experiment. For the sequential study of the changes in the subcutaneous tissue, 2 animals were killed 24 hours after the first and every 4th injection up to the termination of Experiments 1 and 2.

In Experiments 3 and 5, groups of 2 rats were killed at intervals of 4 injections up to the 20th and subsequently after every 8th injection. The subcutaneous tissue at the injection site was removed, fixed and prepared for histological and ultrastructural examination as described previously (Hooson and Grasso, 1971). At the same time intervals, 2 animals in each group received no further injections in order to observe the long-term effects of different numbers of injections. These animals were killed at the termination of the experiment or earlier if tumours developed. The injection site was removed, fixed and prepared for histological examination as previously mentioned. Ten male and 10 female rats were injected subcutaneously twice weekly with 0.5% MNU and 0.1% NQO for a maximum of 35 weeks in order to observe the neoplastic response (Experiments 4 and 6).

In all cases, animals with tumours were killed when the animals deteriorated in health or ulceration of the tumour threatened. When possible, sarcomata were transplanted into the subcutaneous tissue of 4–6 recipient animals which were killed 6 weeks later. No attempt to transplant adenocarcinomata was made.

RESULTS

Tissue reactions to Light Green SF and Blue VRS

(a) *Rats injected throughout the experimental period.*—The local lesions produced by repeated injections of these colourings (1–15) have been reported in an earlier publication (Grasso and Golberg, 1966*a*).

The principal features of these lesions consisted of destruction of the normal architecture of the rat subcutaneous tissue and its replacement by scar tissue. This scar tissue was made up of thick

TABLE I.—*Details of Animals, Treatment Regimen and Tumour Incidence of Experiments Described in the Text*

Expt No.	Species	Strain	Chemical	Solution injected			No. of animals		Tumours			
				Vol ml	Concn %	Frequency × weekly	Initially	At first appearance of tumours	Sarcomata†	No. of sarcomata transplanted successfully	Mammary adenocarcinomata	M
1	Rat	Ash/CFE	Blue VRS	0.5	2	2	132	98	21	6	0	0
2	Rat	Ash/CFE	Light Green SF	1.0	2	2	126	92	29	—*	0	0
3	Rat	Ash/CFE	MNU	0.1	0.5	2	80	64	18	8	4	21
4	Rat	SFF/CSE	MNU	0.1	0.5	2	20	19	11	—*	0	8
5	Rat	CH3/CFE	NQO	0.2	0.1	2	70	54	24	13	0	14
6	Rat	Porton/Wistar	NQO	0.2	0.1	2	20	16	10	—*	0	4

* No transplantations attempted.

† No sex or strain difference observed.

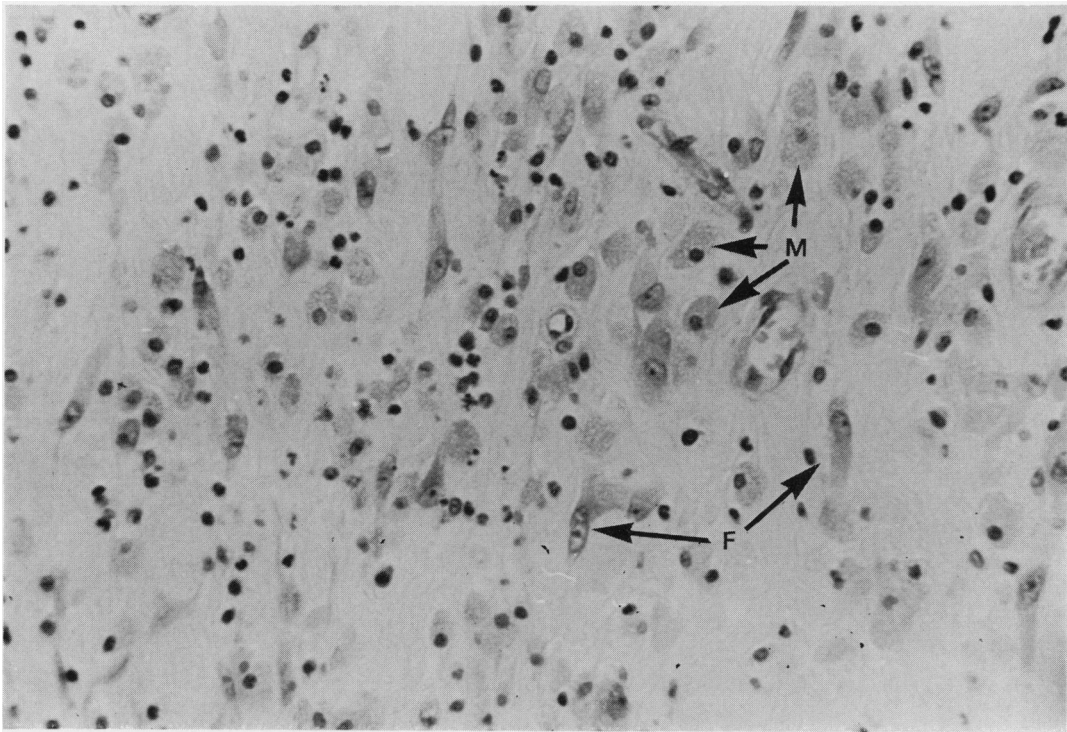


FIG. 1.—Fibroblasts (f) and macrophages (m) containing ingested colouring amongst collagen fibres at the site of 20 injections of Light Green SF. H & E \times 300.

collagen bands, few capillaries and a variable number of fibroblasts and macrophages, the latter generally containing ingested colouring (Fig. 1). Areas of fibroblastic proliferation were often present in the thick collagen (Fig. 2).

The character of the lesion remained essentially the same up to the 60th–70th injection, with few alterations. However, the amount of collagen progressively increased so that on palpation the injection site was considerably thickened. Fewer capillary structures were found in these later lesions so that the scar tissue presented an avascular appearance. The population of fibroblasts was sparse and consisted mainly of small spindle cells (Fig. 3). In a proportion of the sites examined there occurred foci of fibroblasts with large conspicuous nuclei and abundant cytoplasm. The morphological characteristics of these fibroblasts corres-

ponded to the description in the literature (Chapman, 1962; Ross, 1968) of young proliferating and synthesizing fibroblasts. Ultrastructural examination revealed that such cells had abundant, extensive, rough endoplasmic reticulum, with large groups of attached ribosomes, randomly located Golgi and large mitochondria with extensive cristae. The nuclei were large with prominent but structurally normal nucleoli (Fig. 4). Frequent mitotic figures were observed in these foci, indicative of proliferative activity.

Other features less commonly encountered were the formation of cysts containing an eosinophilic proteinaceous exudate and foci of inflammatory cell infiltration, chiefly lymphocytic in character.

Malignant tumours were seen microscopically after 65–70 injections in a proportion of the animals. These were small lesions and consisted of an interlac-

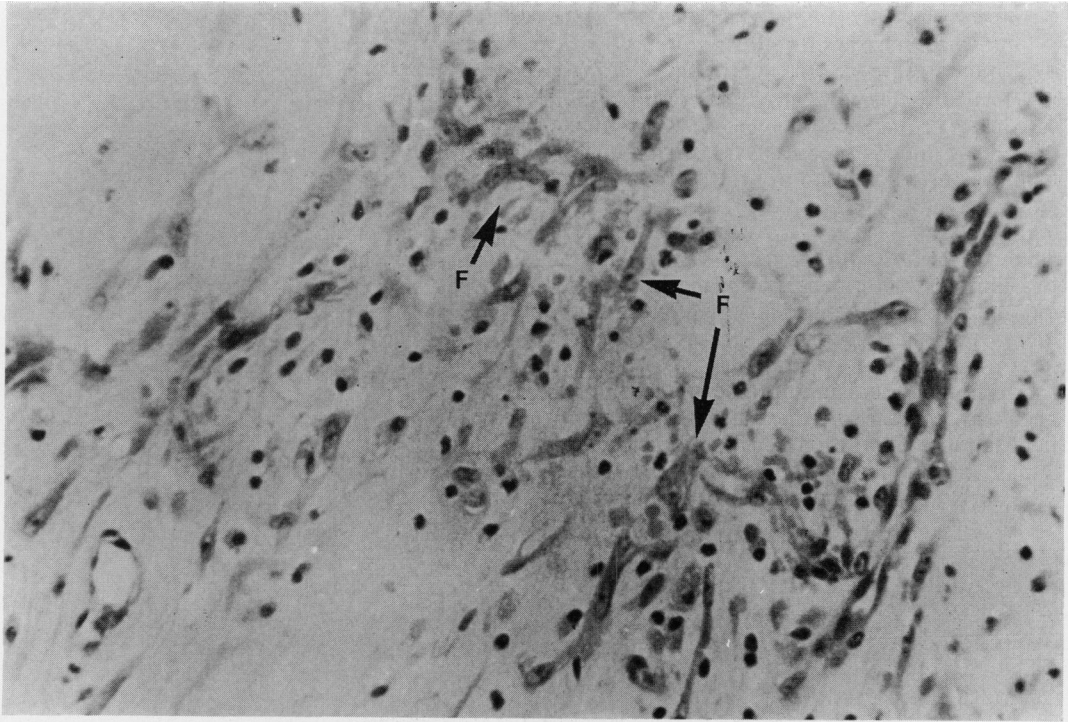


FIG. 2.—Foci of proliferating fibroblasts (f) in heavily collagenized subcutaneous tissue, given 53 injections of Blue VRS. H & E $\times 300$.

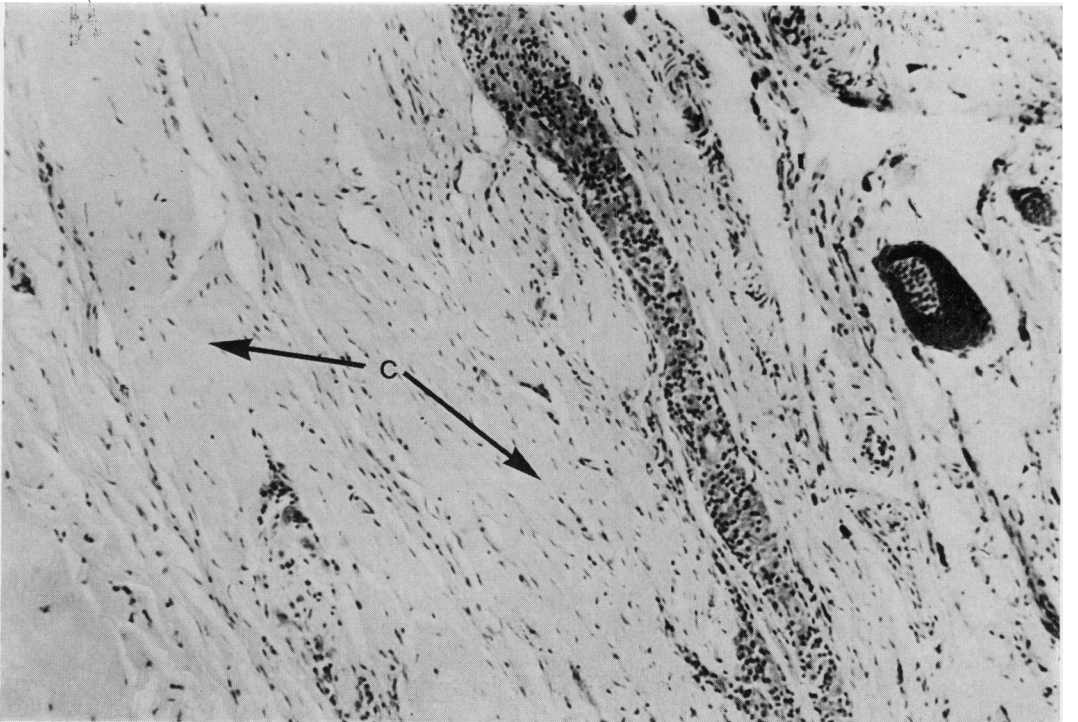


FIG. 3.—Quiescent heavy collagenization (c) of the subpannicular connective tissue layer at the site of 60 injections of Blue VRS. H & E $\times 120$.

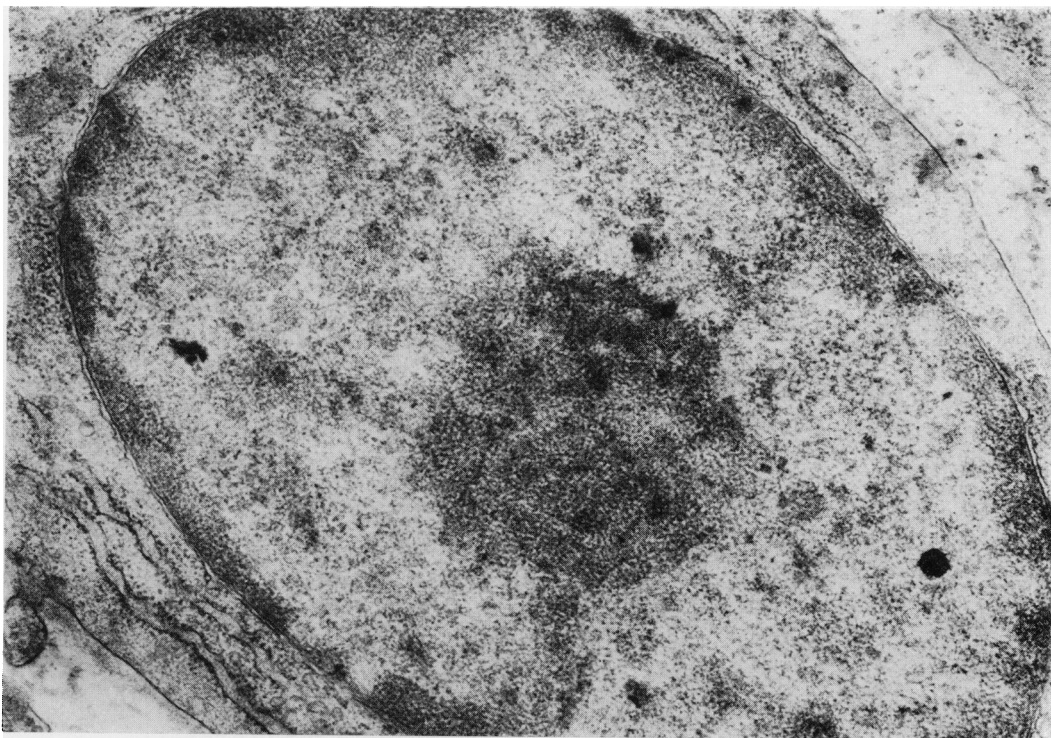


FIG. 4.—Electron micrograph of fibroblast from site of 40 injections of Blue VRS in rats, showing morphologically normal nucleus and nucleolus. Pb citrate/uranyl acetate $\times 30,000$.

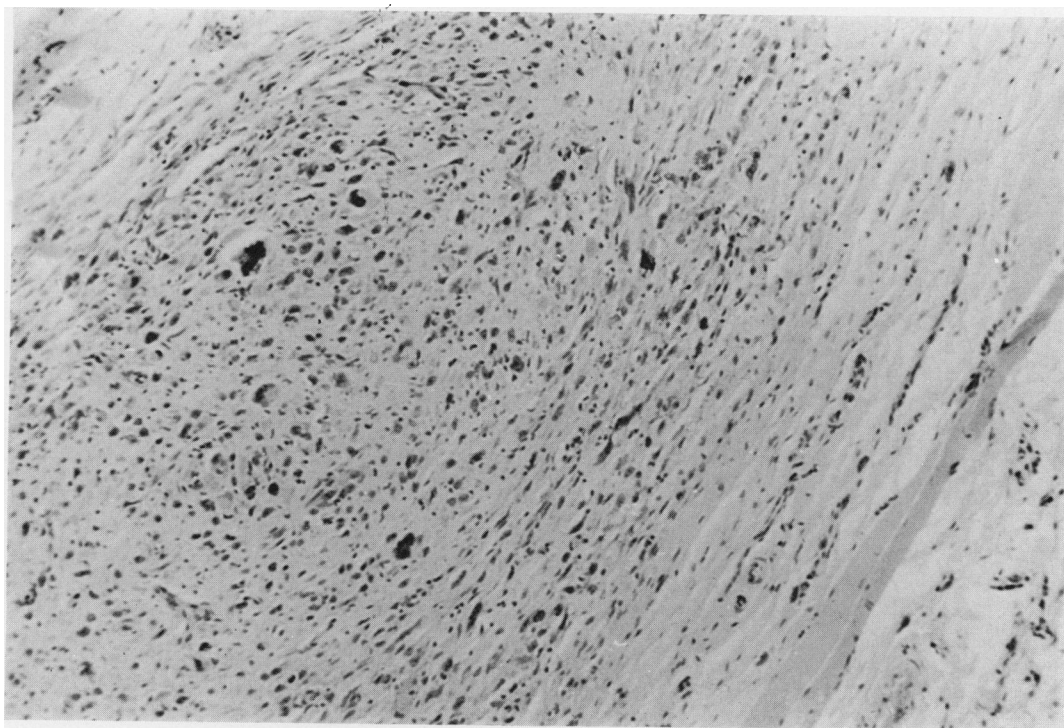


FIG. 5.—Morphologically abnormal fibroblasts, indicative of early sarcomatous change, at the site of 65 injections of Light Green SF. H & E $\times 120$.

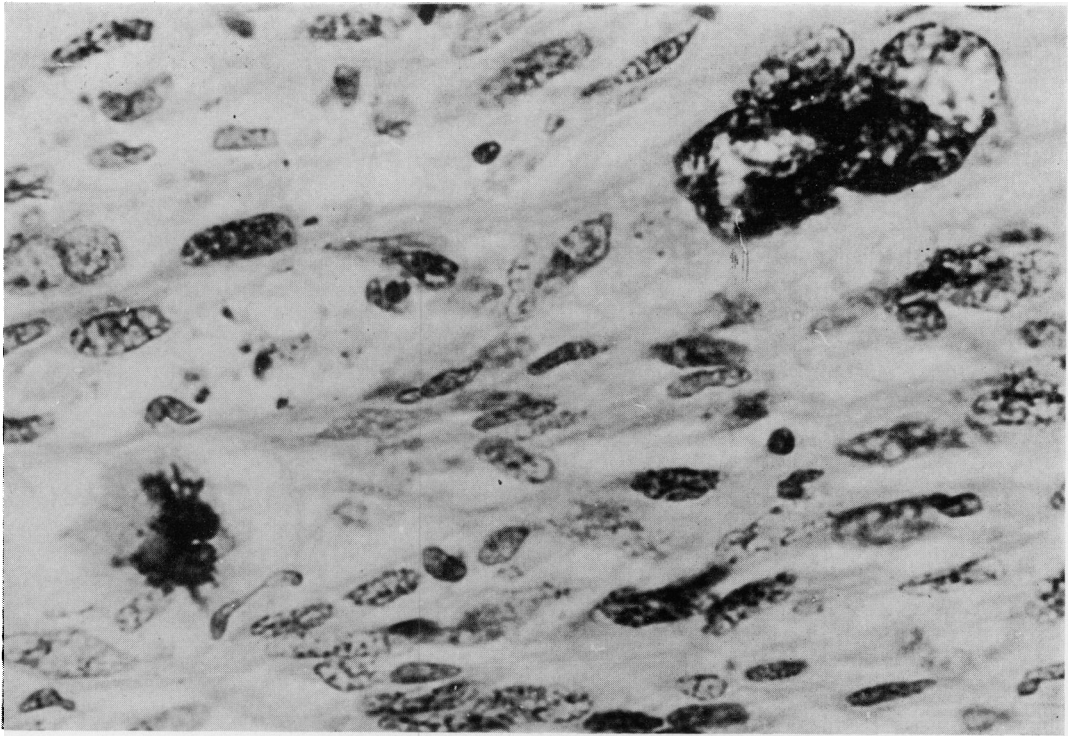


FIG. 6.—Injection site sarcoma in a rat given 90 injections of Blue VRS. H & E $\times 720$.

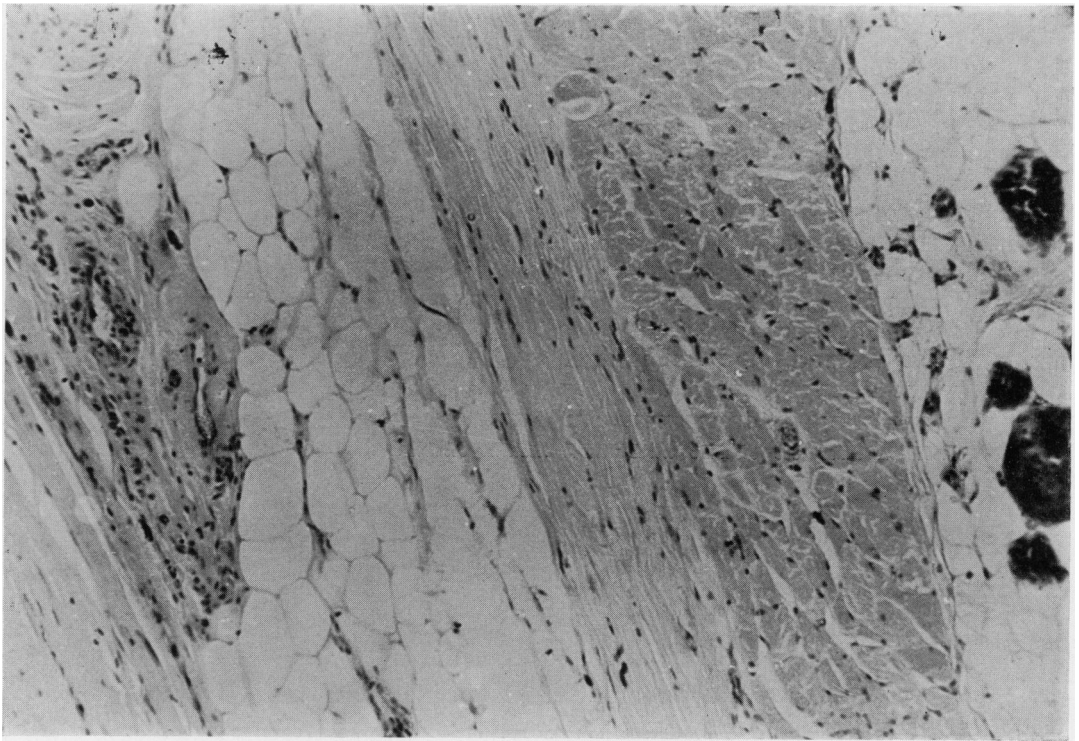


FIG. 7.—Resolution of scar tissue in rat given 20 injections of Light Green SF and allowed to survive until the termination of the experiment. H & E $\times 120$.

ing network of spindle cells, amongst which morphologically abnormal cells were present (Fig. 5). This histological picture conformed to the early sarcomata described by Carter (1969). In some instances there was evidence of invasion of adjacent muscle, establishing beyond doubt the malignant nature of these tumours from an early stage.

In rats that had received more than 70 injections the histology of all sites examined could be fitted into one of 3 categories. The first consisted of those lesions which were composed of extensive collagenization and foci of fibroblastic proliferation. The second category of lesions exhibited areas of early sarcomatous change microscopically and in the third category frank sarcomata were present (Fig. 6) macroscopically forming nodular masses 2–4 cm in diameter.

(b) *Rats injected for only part of the experimental period.*—Animals given up to 25 injections of Light Green SF or Blue VRS and then allowed to survive until the termination of the experiment showed no macroscopical abnormality at the injection site. Histologically, no difference could be detected between these samples and a control uninjected site. Evidently the scar tissue produced in response to the early injections had resolved (Fig. 7).

No resolution was observed in any rat that had received more than 25 injections, although areas of fatty tissue could be seen between the collagen strands in animals receiving less than 40 injections.

In rats that had received more than 40 injections thick scar tissue containing wide collagen bands filled the subcutaneous area, and foci of fibroblastic activity, similar to those described in the earlier section, were seen in several animals. Other animals had developed sarcomata locally. At all stages of the experiment, a heavy macrophage response was evident after injections of Light Green SF. This response was less conspicuous in the lesions induced by Blue VRS.

The principal pathological findings in these experiments are summarized in Table II.

Tissue reaction to carcinogens

(a) *Rats injected throughout the experimental period.*—The initial responses of the subcutaneous tissue to the injection of the carcinogenic agents MNU and NQO have been reported earlier (Hooson and Grasso, 1971). The lesions seen in rats given up to 24 injections did not differ markedly from these. Extensive destruction of the subcutaneous tissue was evident and the site was filled mainly with a haemorrhagic exudate in which fibrinoid fibrillar material containing occasional histiocytes and polymorphs were present (Fig. 8). Typical granulation tissue was absent but around the area of destruction some fibroblastic proliferation was taking place; a few capillary sprouts were in evidence. The fibroblasts showed extensive variation in size and shape and an abnormal nuclear morphology was often observed (Fig. 9).

An early sarcoma was seen histologically in a rat that had received 40 injections. After the 45th injection the majority of the injection sites were found to contain tumours of either the connective tissue or the mammary gland tissue (Fig. 10). In the remaining few animals, the histology did not differ from that described above. Mammary ducts showed some degree of hyperplastic activity, exhibited by increased mitotic activity, and the formation of several layers of epithelial cells at irregular intervals along the lining of the dilated ducts from the 24th injection onwards.

(b) *Rats injected for only part of the experimental period.*—Only 12 of the 55 rats in this group did not bear neoplasms at the injection site. Animals in this series conspicuously developed tumours after very short exposure to carcinogens. For example, adenocarcinomata and sarcomata were recorded 35–41 weeks after the injection of only 4 doses of NQO. No local thickening was observed at the

TABLE II.—*Incidence of Local Tissue Responses Elicited by Surfactants in Rats after Increasing Numbers of Injections (Experiments 1 and 2)*

	No. of injections	Total rats examined	Appearance of subcutaneous tissue in treated rats					Sarcomata (including microscopic)
			Resolution	Quiescent scar tissue	Foci of fibroblastic activity in scar tissue			
<i>Blue VRS</i>								
(a) On treatment*	1-24	12	0	0	6†	0	0	
	32-52	14	0	4	10	0	0	
	56-76	12	0	1	10	1	1	
	80-96	11	0	1	8	2	2	
	100-117	11	0	0	3	8	8	
(b) Off treatment†	1-28	15	15	0	0	0	0	
	32-60	15	0	9	5	1	1	
	64-92	15	0	5	4	6	6	
	96-117	17	0	4	10	3	3	
<i>Light Green SF</i>								
(a) On treatment*	1-20	14	0	0	8†	0	0	
	24-44	14	0	0	14	0	0	
	48-76	14	0	2	11	1	1	
	80-92	13	0	0	7	6	6	
	96-112	24	0	0	20	4	4	
(b) Off treatment†	1-24	11	11	0	0	0	0	
	28-52	15	0	6	2	7	7	
	56-76	11	0	1	3	7	7	
	80-96	10	0	0	6	4	4	

* Rats injected throughout experimental period and killed 24 hours after last injection.

† Rats injected for only part of the experimental period and killed at termination of experiment or when tumours developed.

‡ Other animals showed acute inflammatory lesions.

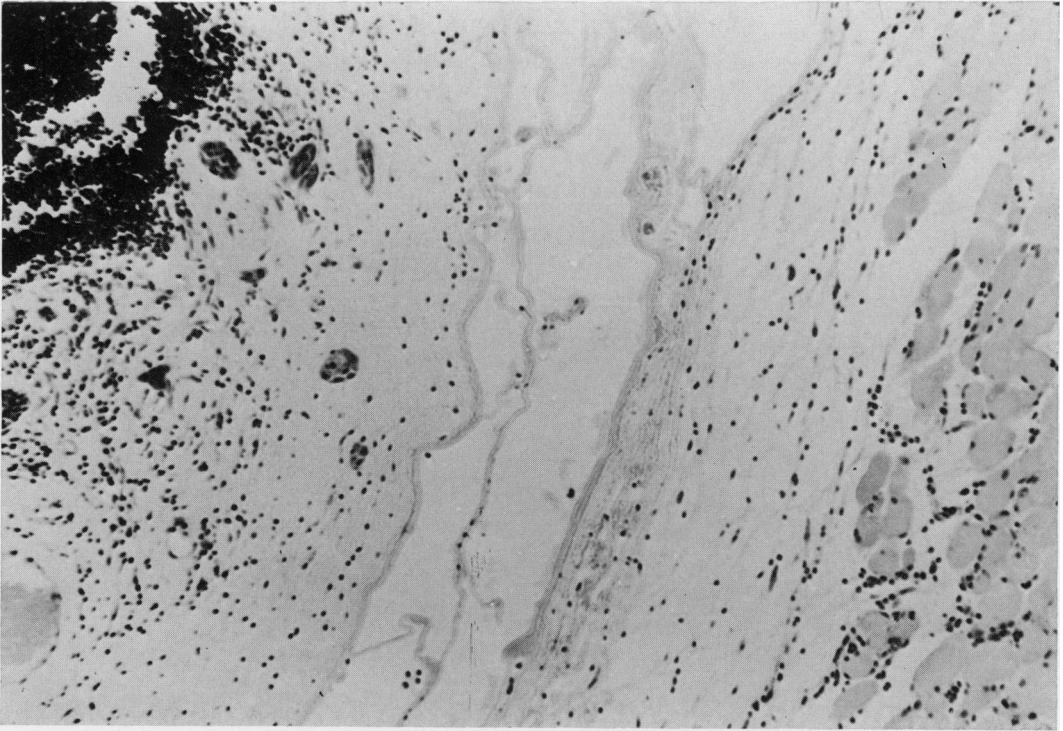


FIG. 8.—Absence of granulation tissue formation or reparative processes operative in the subcutaneous site after 20 injections of MNU in rats. H & E $\times 120$.

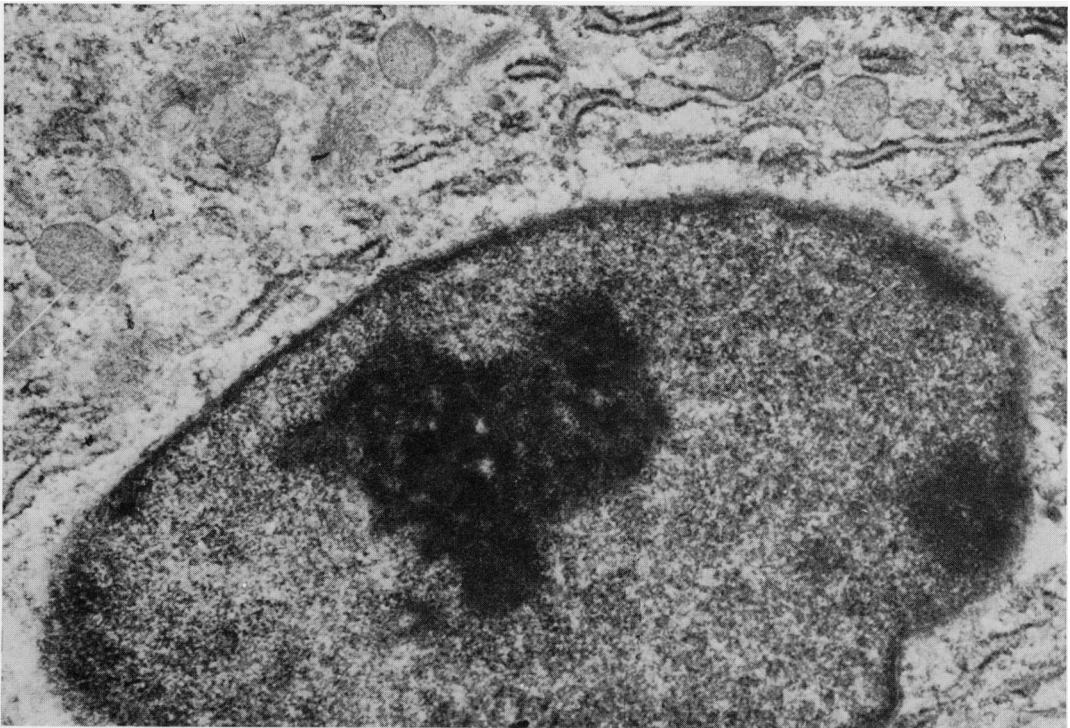


FIG. 9.—Electron micrograph of fibroblast from site of 20 injections of NQO in rats. The nucleus is dense and segregation of nucleolar elements is obvious. Pb citrate/uranyl acetate $\times 25,000$.

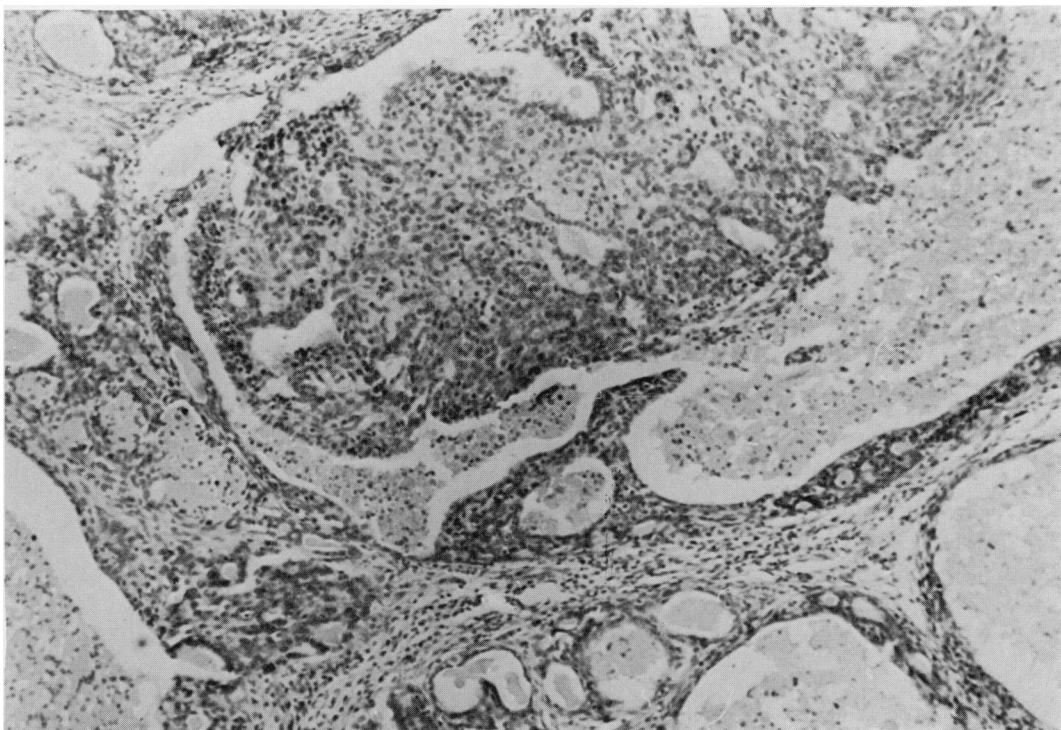


FIG. 10.—Cystic mammary adenocarcinoma at the site of 40 injections of methylnitrosourea in a rat.
H & E $\times 120$.

injection site unless sarcomata were present. Histologically, the injection site contained a thin layer of connective tissue with normal-looking fibroblasts and a slight cellular infiltrate of mononuclears and histiocytes.

The principal pathological findings in these experiments are summarized in Table III.

Induction of tumours

In Experiments 4 and 6, designed specifically to assess the tumorigenic response, 19 out of 20 rats treated with MNU developed local tumours (11 sarcomata and 8 mammary adenocarcinomata). The adenocarcinomata occurred predominantly in females, SPF/CSE females being particularly susceptible.

Of the 16 rats which survived to tumour bearing age after injection of NQO, 10 developed local sarcomata and 4 carried mammary tumours (Table I).

Morphology of injection site tumours

The sarcomata consisted histologically of interlacing bundles of spindle cells. The bundles varied in thickness from a few to several cells. In the differentiated tumours the bundles were clearly defined and were made up of cells closely resembling young immature fibroblasts. Intra-cellular matrix was abundant and consisted of strands of reticulin and collagen. The resemblance of cells and fibres to connective tissue became more indistinct with the progressive loss of differentiation until the tumour cells lost completely their resemblance to the cell of origin and displayed considerable pleomorphism. Despite this range of histological appearance, all the tumours were locally infiltrative, destroying surrounding muscle and overlying skin. No metastases were found.

The mammary adenocarcinomata were either solid or papillary. The solid type consisted of areas packed with small

TABLE III.—*Incidence of Local Tissue Responses Elicited by Carcinogens in Rats after Increasing Numbers of Injections (Experiments 3 and 5)*

MNU	No. of injections	Total rats examined	Appearance of subcutaneous tissue in treated rats					Mammary adenocarcinomata	
			Resolution	Quiescent scar tissue	Abnormal fibroblastic response	Sarcomata	Mammary adenocarcinomata		
(a) On treatment*	1-15	16	0	0	11	0	0	0	
	20-44	16	0	0	4	1	10		
	52-68	12	0	0	2	7	3		
	1-15	8	2	0	0	1	5		
(b) Off treatment†	20-44	15	0	5	0	7	3		
	52-68	7	0	0	2	2	3		
NQO	(a) On treatment*	1-15	0	0	14†	0	0	0	
		20-44	0	0	2	4	8		
	(b) Off treatment†	52-68	14	0	0	11	3		
		1-15	8	4	0	2	2		
	20-44	9	0	1	5	1			
	52-68	2	0	0	0	2	0		

* Rats injected throughout experimental period and killed 24 hours after last injection.
 † Rats injected for only part of experimental period and killed at termination of experiment or when tumours developed.
 ‡ Other animals showed acute inflammatory lesions.

acini or covered with sheets of epithelial cells. The cells were small with a prominent nucleus and displayed a variable amount of pleomorphism and mitotic activity. Again, no metastases were found in this experiment.

DISCUSSION

The results presented in this paper demonstrate that the difference in histological response between the injections of surfactants and carcinogens extends from the first few weeks through to the development of tumours. The resultant neoplastic responses are also in strong contrast to each other, as described later.

Tissue reaction to surfactant colourings

The essential histological findings of progressive collagenization and continued proliferative fibroblastic response in the above experiments with surfactants have also been described at the site of repeated injection of hypertonic glucose (Takizawa, 1940; Cappellato, 1942) in the connective tissue envelope that develops around plastic films (Oppenheimer *et al.*, 1959) and after administration of Fe dextran (Baker *et al.*, 1961) or NTDQ, a rubber additive (Carter, 1969).

In these cases, the incidence of sarcomata recorded is comparable with the high incidence of sarcomata observed in our experiments with the surfactant colourings. Progressive fibroblastic proliferation seems to be a vital factor in the development of these sarcomata.

Carter, Birbeck and Roberts (1970) stated that enhanced premitotic activity, measured by incorporation of [³H] thymidine, continued for 40 weeks after multiple injections of NTDQ. Previous work in our laboratories has shown that colourings devoid of those physical factors known to induce massive local necrosis produce, when injected subcutaneously, a self-limiting lesion in which the connective tissue acquires histological features of maturity. In long-term tests these did

not give rise to local sarcomata (Grasso and Golberg, 1966b).

It is not easy to explain why a continuous proliferative activity of fibroblasts leads to malignancy. A high cellular turnover is associated with an increase in mutation rate, and therefore with a subsequent higher risk of the emergence of malignant cells (Atwood and Scheinberg, 1958). It is possible that some mutant cells have initially some selective advantage over normal fibroblasts, enabling them to survive rather than be eliminated as would be most mutants (Vasiliev *et al.*, 1962). However, other factors also can play a part in the development of neoplasia. Several investigations have stressed the importance of a sustained derangement of the microenvironment in favouring the development of local sarcomata. It is feasible that the thick collagen which develops at the site of the injection could isolate fibroblasts from the regulators of cell growth, such as cell-cell contact, biochemical exchanges and perhaps immunological processes (Carter, 1969).

A similar formation of hyalin collagen is seen when implants of solid materials are made into the subcutaneous tissue of rats, with sarcomata as the ultimate outcome. Histological descriptions of the connective tissue reaction around silicone rubber implants (Nothdurft, 1961) and various plastic films (Oppenheimer *et al.*, 1959) emphasize the importance of the formation of a thick, avascular connective tissue capsule, with consequent isolation of the fibroblasts, as an essential step in the evolution of malignancy.

It is significant that in the injection experiments reported in the present paper, no tumours arise where the local tissue returns to its normal architecture, for example in rats taken off treatment after up to 24 injections of surfactant. The same phenomenon was observed in sequential studies of the connective tissue lesion that develops around plastic films. If the film was removed surgically after 10 weeks of implantation, complete absorp-

tion of the connective tissue capsule occurs and no sarcomata arise (Druckrey, 1960). If surgical removal is delayed until 20 weeks, the scar tissue persists and sarcomata eventually develop (Oppenheimer *et al.*, 1958).

Reactions to carcinogens

A great contrast to these observations was seen when carcinogens were injected into rats. No proliferative lesions occurred but a totally different response was immediately apparent. In these cases, the intermediate stage of the tissue reaction was an extension of the initial reaction elicited during the first 5 weeks of treatment, and thus the predominant feature was an inhibition of fibroblastic proliferation rather than a stimulation to divide. This damping effect persisted histologically to a stage immediately before the appearance of neoplasia at the site.

Similar findings have been recorded previously by Vasiliev *et al.* (1962) when implanting paraffin pellets containing DMBA subcutaneously. Connective tissue proliferation was inhibited and fibroblastic differentiation was depressed. Other carcinogenic polycyclic hydrocarbons have been reported to inhibit the connective tissue response when injected subcutaneously in oil (Orr, 1939; Shabad, 1935; Wolbach, 1936). In the action of carcinogens, a different mechanism responsible for malignant change seems to be in operation and a reaction between an active form of the chemical and a cellular receptor site responsible for cell growth has been postulated.

Comparison of neoplastic response

1. *Latent period.*—Several other factors deserve consideration when comparing the different responses elicited by carcinogens and surfactant colourings. One of these is the induction period for tumorigenesis. When the colourings were used, 80–90 twice weekly injections were the usual number administered before neoplasia occurred. Other workers have

reported similar time intervals with other compounds producing malignant change by a process of repeated fibroblastic proliferation. Thus, local tumours induced by daily injection of hypertonic glucose in rats and mice appeared by 45–50 weeks (Capellato, 1942) and sarcomata induced by sorbic acid were characterized by a long induction period of 52–82 weeks (Dickens, Jones and Waynforth, 1966, 1968). In contrast, injections of MNU and NQO elicited local sarcomata as early as 19 weeks from the beginning of treatment. This reduced time interval is in accordance with the experience of other workers. Repeated injection of several polycyclic aromatic hydrocarbons resulted in local tumours by 12–20 weeks (Bonser and Orr, 1939). The potencies of MNU and NQO are emphasized when off-treatment animals are taken into consideration. Only 4 injections of NQO and 16 injections of MNU gave rise to eventual malignant connective tissue tumours (Table III). Usually around 60 injections of the surfactant colourings were necessary before sarcomata could be induced locally (Table II).

Dose of chemical

The dose of chemical required to produce a carcinogenic effect is another important distinguishing feature of the types of response under discussion. At the termination of the surfactant experiments 1–1.5 g of dye had been administered to rats. With the carcinogens the dose was much smaller. About 40 mg of MNU and 20 mg of NQO were given to rats. Thus the difference in dosage necessary to induce neoplasia is considerable.

Mammary tumour production

A completely new tumorigenic response was elicited by MNU and NQO which has no parallel in the experiments with Light Green SF and Blue VRS. A high percentage of mammary tumours were produced locally, the majority in female rats. Again, a short latent period of 12–13 weeks

characterized the formation of these tumours and very small doses of both carcinogens (4-6 injections) were shown to induce them.

CONCLUSION

It appears that there are certain clear cut differences in the quality of the neoplastic response which, when taken in conjunction with the early and intermediate histological changes at the site of injection, render it possible to distinguish between sarcoma production denoting a true carcinogenic response and sarcomata arising from a derangement of connective tissue repair.

REFERENCES

- ATWOOD, K. C. & SCHEINBERG, S. L. (1958) Somatic Variations in Human Erythrocyte Antigens. *J. cell. comp. Physiol.*, Suppl. 1, 97.
- BAKER, S. B. DE C., GOLBERG, L., MARTIN, L. E. & SMITH, J. F. (1961) Tissue Changes Following Injection of Iron-Dextran Complex. *J. Path. Bact.*, **82**, 453.
- BONSER, G. M. & ORR, J. W. (1939) The Morphology of 160 Tumours Induced by Carcinogenic Hydrocarbons in the Subcutaneous Tissues of Mice. *J. Path. Bact.*, **49**, 171.
- CAPELLATO, M. (1942) Sui sarcomi sperimentali da glucosio nel ratto bianco. *Tumori*, **16**, 38.
- CARTER, R. L. (1969) Early Development of Injection Site Sarcomas in Rats; a Study of Tumours Induced by a Rubber Additive. *Br. J. Cancer*, **23**, 408.
- CARTER, R. L., BIRBECK, M. S. C. & ROBERTS, J. D. B. (1970) Development of Injection Site Sarcomata in Rats; a Study of the Early Reactive Changes Evoked by a Carcinogenic Nitrosoquinoline Compound. *Br. J. Cancer*, **24**, 300.
- CHAPMAN, J. A. (1962) Fibroblasts and Collagen. *Br. med. Bull.*, **18**, 233.
- DICKENS, F., JONES, H. E. H. & WAYNFORTH, H. B. (1966) Oral, Subcutaneous and Intratracheal Administration of Carcinogenic Lactones and Related Substances: the Intratracheal Administration of Cigarette Tar in the Rat. *Br. J. Cancer*, **30**, 134.
- DICKENS, F., JONES, H. E. H. & WAYNFORTH, H. B. (1968) Further Tests on the Carcinogenicity of Sorbic Acid in the Rat. *Br. J. Cancer*, **22**, 76.
- DRUCKREY, H. (1960) Berliner Symposium über Fragen der Carcinogenese. *Abh. dt. Akad. Wiss. Berl., Klasse Med.*, **3**, 98.
- GRASSO, P., GANGOLLI, S. D., GOLBERG, L. & HOOSON, J. (1971) Physico-chemical and Other Factors Determining Local Sarcoma Production by Food Additives. *Fd. Cosmet. Toxicol.*, **9**, 463.
- GRASSO, P. & GOLBERG, L. (1966a) Early Changes at the Site of Repeated Injection of Food Colourings. *Fd. Cosmet. Toxicol.*, **4**, 269.
- GRASSO, P. & GOLBERG, L. (1966b) Subcutaneous Sarcoma as an Index of Carcinogenic Potency. *Fd. Cosmet. Toxicol.*, **4**, 297.
- HOOSON, J. & GRASSO, P. (1971) Early Reactions of the Subcutaneous Tissue to Repeated Injections of Carcinogens in Aqueous Solutions. *Br. J. Cancer*, **25**, 505.
- NOTHDURFT, H. (1961) Sarkomergengung bei Ratten durch implantierte Fremdkörper. *Ther. mh.*, **8**, 262.
- ORR, J. W. (1939) An Investigation of the Histological Changes of the Subcutaneous Tissues of Mice during the Induction of Sarcoma by Carcinogenic Hydrocarbons. *J. Path. Bact.*, **49**, 157.
- OPPENHEIMER, B. S., OPPENHEIMER, E. T., STOUT, A. P., WILLHITE, M. & DANISHEFSKY, I. (1958) The Latent Period in Carcinogenesis by Plastics in Rats and its Relation to the Precancerous Stage. *Cancer, Philad.*, **11**, 204.
- OPPENHEIMER, B. S., OPPENHEIMER, E. T., STOUT, A. P., DANISHEFSKY, I. & WILLHITE, M. (1959) Studies on the Mechanism of Carcinogenesis by Plastic Films. *Acta Un. int. Canc.*, **15**, 659.
- ROSS, R. (1968) The Fibroblast and Wound Repair. *Biol. Rev.*, **43**, 51.
- SCHABAD, L. M. (1935) Über die cancerogene Wirkung des 1 : 2 : 5 : 6 Dibenzanthracene. *Z. Krebsforsch.*, **42**, 295.
- SHELTON, E., EVANS, V. J. & PARKER, G. A. (1963) Malignant Transformation of Mouse Connective Tissue Grown in Diffusion Chambers. *J. natn. Cancer Inst.*, **30**, 377.
- TAKIZAWA, N. (1940) Experimentelle Erzeugung des Sarkoms bei der Maus durch die Injektion von Glucose, Fructose und Galactose. *Gann.*, **34**, 1.
- VASILIEV, J. M., OLSHEVSKAJA, L. V., RAIKHLIN, N. T. & IVANOVA, O. J. (1962) Comparative Study of Alterations Induced by 7,12-dimethylbenz(a)anthracene and Polymer Films in the Subcutaneous Connective Tissue of Rats. *J. natn. Cancer Inst.*, **28**, 515.
- WOLBACH, S. B. (1936) The Latent Period in Experimental Carcinogenesis. *Archs Path.*, **22**, 279.