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Preliminary Survey of 22 Printing Inks for Carcinogenic Activity by the Subcutaneous Route in Mice

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Abstract—A preliminary study of 22 printing inks for carcinogenic activity by the subcutaneous route has been conducted in mice.

A total of 440 male CB stock mice received 15–22 weekly subcutaneous injections of one of 22 printing inks, either dissolved in distilled water or suspended in arachis oil. In addition, 40 mice were injected with distilled water alone, 40 mice received arachis oil only and 40 mice were given no treatment. Test and control animals were observed for up to 2 yr.

Local sarcomas developed in one mouse in each of five test groups. In two of these five groups, metastasizing adenocarcinomas of the lung were also seen. No injection-site neoplasms were found in the remaining 17 test groups and the incidence and types of distant neoplasms were similar to those in mice from the three control groups. The five inks which produced local tumours are to be examined in more detail.

INTRODUCTION

The occupational hazards associated with printing have received comparatively little attention. Among non-neoplastic conditions, there have been reports of a raised incidence of pulmonary tuberculosis (Lloyd Davies, 1957), asthma (Harvey & Murray, 1958) and contact dermatoses (Schwartz, 1958). But Ask-Upmark (1955) found that a group of typographers exposed to printing inks in Stockholm had an unusually high incidence of bronchogenic carcinoma—18 times greater than that recorded in the general population of Stockholm. Furthermore, some of the printers with lung cancer were less than 40 yr old when the disease was diagnosed. This report is difficult to interpret as information on smoking habits was not recorded, but the findings are disturbing, particularly as there is some evidence that printing inks may induce tumours in experimental animals. Steinbrück (1929, 1930) applied printing ink to the dorsal skin of 16 mice and subsequently observed malignant tumours in eight of them. Five of the mice developed epitheliomas of the painted skin (some of which metastasized to the lungs) and three animals developed lymphomas.

Most printing inks are mixtures of dyes and other compounds; many of them are not chemically characterized and, in some instances, their composition is continually being modified. Because of these difficulties and because there was no guide from previous studies with regard to which inks deserved closest scrutiny, we selected for test 22 inks more or less at random and without knowledge of their chemical constitutions.

EXPERIMENTAL

Over a period of several months, 560 male CB stock mice, aged 11 wk, were used. Groups of 20 mice were given courses of subcutaneous injections of one of 22 different printing inks (Table 1), dissolved or suspended in water or arachis oil. Forty control animals were injected with distilled water and 40 with arachis oil, while 40 received no treatment. Because the experiments were spread over several months, test and control groups were not strictly comparable; but experience of this strain of mouse in similar experiments over a period of many years has been so consistent, that the development of neoplasms at the site of injection can with confidence be attributed to the material injected, even in the absence of data from strictly comparable control groups. Mice were housed in metal cages, five in each, and were fed a cubed diet (E. Dixon & Sons (Ware) Ltd., Ware, Hertfordshire) and water *ad lib.*

Table 1. *Summary of experimental details*

	Printing ink	Vehicle for injection	Injection volume (ml)	No. of weekly subcutaneous injections	No. of mice used
No.	Name and source				
		Test groups			
1	Process Black L/Press B1055*	Distilled water	0·5	15	20
2	L/Press Cigarette Black 4†	Arachis oil	0·5	20	20
3	Bronze Blue L/P Ref. S/P 1/20‡	Distilled water	0·5	21	20
4	Scarlet Lake Ref. 0141§	Distilled water	0·2	20	20
5	Bronzing medium CO 901§	Distilled water	0·2	15	20
6	L/P Blue Ref. 353†	Arachis oil	0·2	20	20
7	Vivid Green Ref. CD 41172 & CX 11026§	Arachis oil	0·2	20	20
8	Bronzing Medium Ref. B/62979‖	Distilled water	0·2	20	20
9	Concentrated Brown Ref. H/1651¶	Arachis oil	0·2	20	20
10	Blue L/Press Ref. CM 41870§	Arachis oil	0·2	22	20
11	Fadeless Blue Ref. CM 21798§	Arachis oil	0·2	20	20
12	Burnt Amber Ref. CX 14207 & CD 10101/CO 322§	Distilled water	0·2	20	20
13	Dark Milori Blue Ref. CD 41171§	Distilled water	0·2	20	20
14	Brown No. 3 Ref. F/1254¶	Arachis oil	0·2	20	20
15	Red L/P Ref. C 49535§	Distilled water	0·2	20	20
16	Blue Black Ref. 84753§	Arachis oil	0·2	20	20
17	L/P Black Ref. 0361§	Distilled water	0·2	20	20

TABLE 1 *cont.*

Printing ink		Vehicle for injection	Injection volume (ml)	No. of weekly subcutaneous injections	No. of mice used
No.	Name and source				
18	Medium Sepia Ref. F/1541¶	Distilled water	0·2	20	20
19	Special Blue Ref. 5¶	Distilled water	0·2	19	20
20	Bronze Blue Ref. SPl‡	Distilled water	0·2	20	20
21	L/P Blue Ref. CX 27291§	Distilled water	0·2	21	20
22	L/P Green Ref. S 445‡	Distilled water	0·2	21	20
		Control groups			
		Distilled water only	0·2	80	40
		Arachis oil only	0·2	80	40
		Untreated	—	—	40

* Usher-Walker Ltd., London.
† Fishburn & Co. Ltd., Watford, Herts.
‡ Winstone Ltd., Heathfield, Middx.
§ Mander-Kidd Ltd., London.
‖ Lorilleux & Bolton Ltd., London.
¶ Forrest Printing Ink Company, London.

A total of 15–21 weekly subcutaneous injections of each ink was given into the right flank. The dose, vehicle, number of injections and number of animals treated are shown in Table 1. The proportion of each undiluted dye in the volume injected was unknown.

The mice were examined regularly. Sick animals were killed with ether vapour and the survivors were killed when they were approximately 2 yr old. Post-mortem examinations were carried out and tissues showing macroscopic abnormalities were fixed in Bouin's solution. Paraffin sections were prepared at 5 μ and stained with haematoxylin and eosin for histological examination.

RESULTS

Survival rates in test and control mice are shown in Table 2. Except for the poor survival in mice injected with printing ink no. 18, the lifespan of mice in the test and control groups was comparable. No toxic effects were observed with any of the inks examined.

Repeated subcutaneous injections of 17 of the inks failed to induce local tumours and the types and incidence of neoplasms at other sites did not differ from that found among the control animals. The most common neoplasms were hepatomas, lymphomas and pulmonary adenomas; no carcinomas of the lung were seen in these mice.

Treatment with five of the printing inks was associated with the development of sarcomas at the site of injection; the induction times of these lesions are shown in Table 2. The first

Table 2. *Mortality rate and incidence of local tumours in male mice given 15–21 weekly subcutaneous injections of 22 printing inks*

Test or control injections	No. of survivors at month					Local tumours	
	0*	6	12	18	24	No.	Induction time (months after first injection)
Test groups							
Printing ink no.†							
1	20	17	15	12	1	1	18
2	20	20	17	12	3	0	—
3	20	19	13	11	2	1	19
4	20	20	16	7	0	0	—
5	20	17	16	9	2	0	—
6	20	20	15	8	2	0	—
7	20	20	15	10	2	0	—
8	20	20	19	13	2	0	—
9	20	20	16	11	1	1	19
10	20	20	17	12	4	0	—
11	20	17	16	8	0	0	—
12	20	19	17	9	1	0	—
13	20	20	17	12	6	0	—
14	20	20	18	12	1	1	18
15	20	20	17	10	1	0	—
16	20	18	16	9	0	0	—
17	20	20	17	11	1	0	—
18	20	18	9	0	—	1	7
19	20	20	20	12	5	0	—
20	20	19	12	11	2	0	—
21	20	20	15	14	3	0	—
22	20	19	17	7	1	0	—
Control groups							
Distilled water	40	40	35	24	5	0	—
Arachis oil	40	38	32	21	3	0	—
None	40	35	30	25	1	0	—

* Initial group size.
† The numbers refer to the printing inks listed in full, in the same order, in Table 1.

tumour appeared after only 7 months in a mouse treated with ink no. 18, i.e. in the group in which survival was exceptionally poor. The remaining four tumours, all in different groups, first became palpable between 18 and 19 months. The four late-developing neoplasms were well-differentiated spindle cell lesions, but the tumour which arose at 7 months was more anaplastic (Figs 1 & 2). One epithelial tumour of the skin was encountered, namely an invasive squamous carcinoma which developed on the dorsal skin close to, but not at, the injection site 13 months after the start of treatment with ink no. 6.

The distribution of certain distant tumours in the five groups showing local tumours presented two unusual features which were not seen in any of the other test or control animals. One mouse, injected with ink no. 18, was found at autopsy to have a large retroperitoneal spindle-cell sarcoma; no injection site tumour was present. In addition, two mice (one injected with ink no. 3 and one with ink no. 9) developed metastasizing adenocarcinomas of the lung; one metastasized to mediastinal and lumbar lymph nodes and the

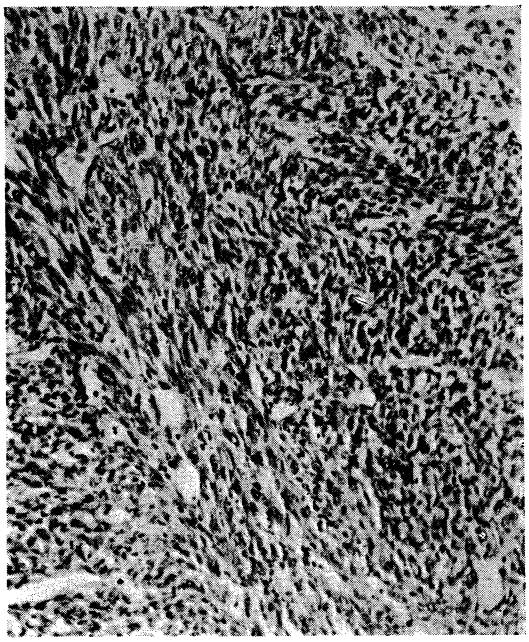

FIG. 1. Well-differentiated spindle-cell sarcoma which developed after 19 months at site of repeated subcutaneous injection of printing ink no. 3 in a male mouse. Haematoxylin and eosin × 200.

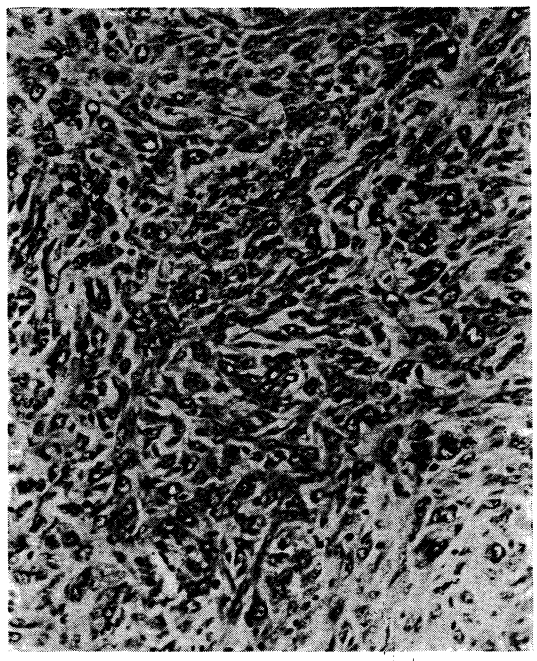

FIG. 2. Poorly-differentiated sarcoma, palpable after 7 months at site of repeated subcutaneous injection of printing ink no. 18 in a male mouse. Haematoxylin and eosin × 200.

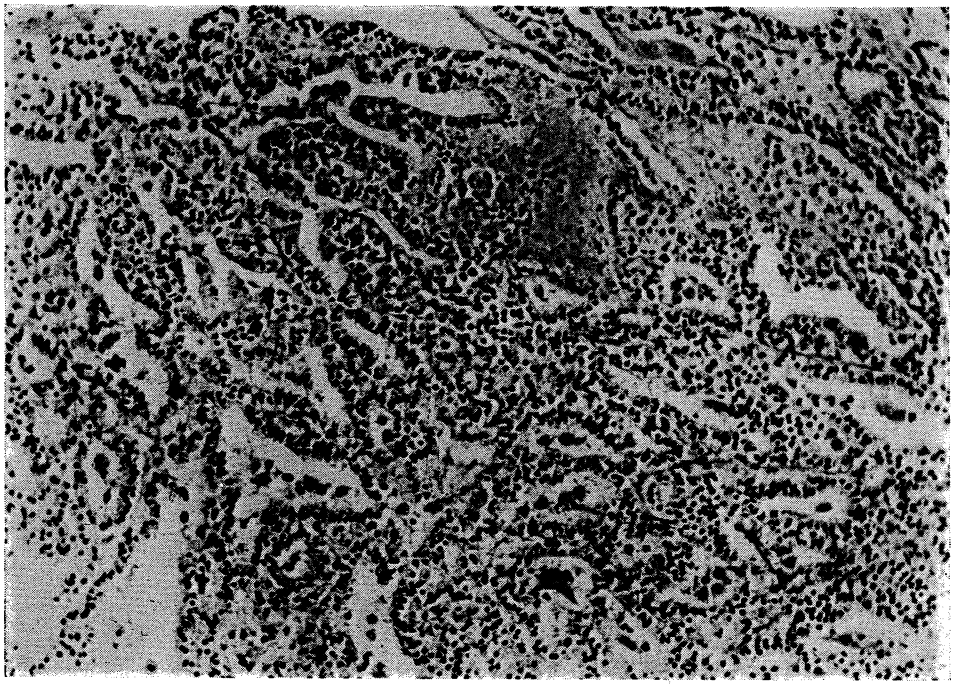

FIG. 3. Secondary deposit in the injection site resulting from a metastasizing papillary adenocarcinoma of the lung, 11 months after repeated subcutaneous injection of printing ink no. 18 in a male mouse. (Animal was found dead and tissues showed early post-mortem autolysis.) Haematoxylin and eosin × 200.

other to the liver and subcutaneous tissues of the flank. Although occurring at an injection site, the subcutaneous deposit clearly differed in histological appearance from the primary sarcomas previously described (Fig. 3). The incidence of pulmonary adenomas was not increased in any of the five groups.

No local neoplasms were seen in mice injected with arachis oil or distilled water and no subcutaneous tumours were found among the group of untreated animals. No tumours of the urinary bladder were seen in any group and the incidence of hepatoma and malignant lymphoma was no higher in any of the treated groups than in the control groups.

DISCUSSION

Of the 22 printing inks tested, 17 proved inactive after 15–22 weekly subcutaneous injections into CB stock mice. Local sarcomas were produced with five of the printing inks but their significance, and particularly that of four tumours which developed after intervals of 18–19 months, is doubtful. Subcutaneous sarcomas occasionally arise spontaneously (Slye, Holmes & Wells, 1917; Dunn, Heston & Deringer, 1956). Furthermore, although no sarcomas were seen in the vehicle-injected control groups in the present experiment, it is well known that local sarcomas can be induced in rats and mice by a variety of substances, some of which are legitimately thought to present no real carcinogenic hazard to man (Grasso & Golberg, 1966). On the other hand, the incidence of spontaneous subcutaneous sarcomas in CB stock mice is low and it is improbable that such lesions, when they occur, should do so solely at injection sites. The poorly-differentiated sarcoma which appeared after only 7 months in a mouse injected with printing ink no. 18 is of particular interest, not only because the latent interval was short, but also because poor survival reduced the chance of seeing tumours in this group. Clearly, further tests with ink no. 18 are indicated.

The occurrence of local tumours is of more significance if the co-existence of neoplasms at other sites is considered. In two of the five test groups that developed local sarcomas, adenocarcinomas of the lung were observed. Such tumours are noteworthy for two reasons: firstly, adenocarcinomas are uncommon in CB stock mice unless, for some reason, there is a preceding increase in the number of pulmonary adenomas in which malignant transformation later takes place; and secondly, both adenocarcinomas metastasized to distant sites. On the few occasions when such tumours disseminate, deposits are usually confined to the chest and their spread to extra-thoracic regions is exceptional (Wells, Slye & Holmes, 1941; Stewart, 1959).

It would be premature to discuss the wider implications of this preliminary survey but it is reassuring that none of the printing inks investigated were found to be potent carcinogens and that none gave rise to neoplasms of either the liver or the bladder. The five inks which were associated with tumours, particularly ink no. 18, will, however, be examined in more detail.

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Etude préliminaire de 22 encres d'imprimerie sous le rapport de l'effet carcinogène exercé par la voie sous-cutanée sur les souris

Résumé—Une étude préliminaire de l'effet carcinogène de 22 encres d'imprimerie par voie sous cutanée a été entreprise sur des souris.

Des souris mâles de souche CB, en tout 440 individus, ont reçu de 15 à 22 injections sous-cutanées hebdomadaires d'une de ces 22 encres d'imprimerie, soit dissoute dans de l'eau distillée, soit en suspension dans de l'huile d'arachide. D'autre part, 40 souris ont reçu des injections uniquement d'eau distillée, 40 uniquement d'huile d'arachide et 40 n'ont subi aucun traitement. Les animaux traités et les animaux témoins ont été observés pendant 2 ans.

Une souris de chacun des 5 groupes d'essai a contracté un sarcome local. Des adénocarcinomes du poumon, avec métastases, ont aussi été décelés dans deux de ces cinq groupes. Aucun néoplasme du site d'injection n'a été constaté dans les 17 autres groupes d'essai; la fréquence et les types de néoplasmes distants dans ces groupes et chez les souris des trois groupes témoins étaient similaires. Les cinq encres qui ont produit des tumeurs locales devront être étudiées plus en détail.

Vorläufige Untersuchung der carcinogenen Wirkung von 22 Druckfarben auf dem subkutanen Weg bei Mäusen

Zusammenfassung—Eine vorläufige Untersuchung der subkutanen carcinogenen Wirkung von 22 Druckfarben wurde an Mäusen durchgeführt.

Insgesamt 440 männliche CB-Mäuse erhielten 15–22 wöchentliche subkutane Injektionen mit 22 Druckfarben, die entweder in destilliertem Wasser aufgelöst oder in Erdnussöl suspendiert waren. Ausserdem erhielten 40 Mäuse Injektionen mit reinem destilliertem Wasser, 40 Mäuse mit reinem Erdnussöl und 40 Mäuse keine Injektionen. Versuchs- und Kontrolltiere wurden bis zu 2 Jahre lang beobachtet.

Örtliche Sarkome entwickelten sich bei je einer Maus von fünf Versuchsgruppen. Bei zwei dieser fünf Gruppen wurden auch metastasierende Adenocarcinome der Lunge beobachtet. Bei den restlichen 17 Versuchsgruppen wurden keine Neoplasmen an der Injektionsstelle gefunden, und die Häufigkeit und die Art entfernt auftretender Neoplasmen waren denen der Mäuse in den drei Versuchsgruppen vergleichbar. Die fünf Druckfarben, die örtliche Tumoren erzeugten, werden noch genauer untersucht.