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EVALUATION OF CARCINOGENIC EFFECT OF MINERAL OIL USED IN THE PROCESSING OF JUTE FIBRES

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Summary.—To evaluate the carcinogenic activity of jute-batching oil (JBO), this substance was painted on the skin of ITRC mice up to 300 days. Initially hyper- and parakeratosis of the stratum corneum, acanthosis and spongiosis of the stratum Malpighii, hyperactivity of fibroblasts, and laying down of collagen fibres in the dermis were encountered at 100 days. This was followed by poor hair growth, acne formation and ulceration. As time passed, these animals partially adapted themselves to the oil-painting so that by 200 days hyperkeratosis and parakeratosis of the stratum corneum, as well as acanthosis and spongiosis of the stratum Malpighii, had almost disappeared. The ulcers healed and no more acne was visible; however, the baldness and loss of hair appendages persisted to 300 days. No carcinogenic changes in the skin or in the viscera of these mice were observed. On UV and IR spectroscopy no traces of any polycyclic aromatic hydrocarbons were found in the JBO sample. Mice, on the other hand, when painted with the known carcinogen 3,4 benzpyrene (BP), developed skin tumours, showing that the mice used in this study were not cancer-resistant. Also, when JBO was applied with BP, the time taken for tumour development in mice was shortened by about 4 weeks as compared to another group painted with the same dose of BP alone. This suggests a cancer-promoting activity which needs to be investigated further.

ONLY a few reports have been published on skin changes in workers associated with manufacture of jute textiles. Curjel and Acton (1924) and Bhar (1972) reported that only oil acne develops in these workers. Henry (1947) described the development of scrotal carcinoma in one out of 1421 notifications to the factory inspectorate. Kinnear *et al.* (1955), however, reported that apart from oil acne, which was a common finding, there was an unusually high incidence of dermatoses; in particular, premalignant degenerative changes occurred with increased frequency and these might progress to overt carcinoma. There was a variety of lesions virtually confined to the exposed areas of skin.

The mineral oil, sprayed on in the form of a water-based emulsion to soften the jute fibres for spinning, has been suspected to be the chief offender. This processing is called "batching" and the oil as jute-batching oil (JBO). Experimental evidence was provided by Harington and Roe (1965) and Roe, Carter and Taylor (1967) that the samples of batching oils studied were carcinogenic when applied to the dorsal skin of mice and that the same material also promoted the development of skin tumours in mice previously exposed to a sub-carcinogenic dose of a known carcinogen. The latter work also incorporated a study of JBO which was shown to contain 3,4 benzpyrene (BP) at just detectable levels.

In order to evaluate a sample of JBO used by jute industries in India for its carcinogenic and/or cancer-promoting properties, the following experimental study was done.

MATERIALS AND METHODS

The whole experiment was performed in 2 phases:

Phase I study

Animals.—Thirty male, adult, healthy, inbred, ITRC strain mice weighing 15–20 g were taken for study. They were kept in groups of 5 in each cage and fed Hindustan Lever Pellet diet; water was provided *ad libitum*. These animals were randomly divided into 2 groups each of 15 animals.

Mineral oil.—Jute-batching oil (JBO) for testing was obtained from the Director, Indian Jute Industries Research Association, Calcutta. This sample was of FDA quality and its physico-chemical characteristics were as follows:

Sp. gravity (at 15·5°)—0·865
Viscosity, C.S. (at 37·8°)—9·0
Flash point—145°
Acid value (mg KOH/g)—0·06
Saponification value—1·0

A sample of JBO was examined for polycyclic aromatic hydrocarbons (PAH) by infra-red and ultraviolet spectroscopy. No evidence of PAH was found.

Bioassay procedure.—An area of 1·5 cm^2 in the interscapular region of each mouse was shaved and cleansed with absolute alcohol in both the groups. Group I (experimental) mice were painted with JBO with the tip of a glass rod 5 mm in diameter dipped in oil. This painting was done thrice weekly in the shaved area. Group II (control) animals were painted with physiological saline in a similar fashion. Painting continued for a total period of 300 days, although phased killing of animals from both the groups was carried out at 100, 200 and finally at 300 days (Table I). Any loss of hair or poor hair growth, acne, ulceration, crust formation or development of tumours were looked for.

Phase II study

Animals.—Forty male, adult, ITRC strain mice of the same weight range and specifications as in the Phase I study were taken for study and kept on the same diet. They were kept in groups of 5 each and divided into 4 groups, each of 10 animals.

JBO.—The same sample as in the Phase I study was used in this study also.

TABLE I.—*Effect of Painting of Jute-batching Oil (JBO) on the Skin of Mice (Phase I Study)*

	Group I (JBO-painted)	Group II (Saline-painted)
Total number of animals taken	15	15
Animals dying during the course of experiment	2	2
Animals killed after 100 days	5	5
Animals killed after 200 days	4	4
Animals killed after 300 days	4	4

3,4 Benzpyrene (BP).—A sample of 3,4 benzpyrene manufactured by M/s Sigma Chemicals Co., U.S.A., was obtained from the Indian Institute of Science, Bangalore, India. It was dissolved in strengths of 5 μg and 50 μg/0·1 ml of AR grade acetone.

Bioassay procedure.—The backs of all mice were shaved, as in the Phase I study. Animals from each group were individually painted with materials as follows:

Group A (Control)—0·1 ml AR grade acetone only.
Group B—0·1 ml AR grade acetone in which was dissolved 50 μg BP.
Group C—0·1 ml AR grade acetone in which was dissolved 5 μg BP.
Group D—0·1 ml AR grade acetone in which was dissolved 5 μg BP followed by 0·03 ml JBO.

These paintings were carried out thrice weekly with a tuberculin syringe and No. 28 needle. Drops were allowed to fall on to the shaved area of the back. Animals were watched for any developments as for the Phase I study. The whole experiment was continued for 43 weeks (301 days) (except for Group B, in which all the surviving 9 animals developed tumours by 20 weeks (140 days) and were killed).

Skin from the painted areas (with or without tumour formation), liver, spleen, stomach, kidneys and urinary bladder were taken, fixed in 10% buffered formalin, and 5μm thick sections cut and stained with haematoxylin and eosin for histopathological studies.

RESULTS

Phase I study

Gross examination.—As shown in Table I, none of the animals belonging to the experimental, *i.e.* JBO-painted group (Group I), or control, *i.e.* saline-painted group (Group II), developed tumours. However, all the existing 8 animals belonging to Group I showed loss of hair and poor hair growth in the oil-painted area by 128 days (approx. 18 weeks). By 146 days (approx. 21 weeks) the loss of hair in the painted areas of these mice was very prominent (Fig. 1), only one out of 8 animals (12·5%) had acne formation and in one (12·5%) small ulcers and crust formation were present. By 175 days (25 weeks) 4 out of 8 mice (50%) developed these ulcers and crusts on their backs, the rest of them showing only marked loss of hair in the

painted and adjoining areas. These ulcers healed subsequently although the painting was continued.

Mice belonging to Group II remained healthy throughout the course of experiment; no loss of hair, acne or ulcer formation was noticed in this group. Two mice belonging to each group died in the early days of the experiment. They were examined *post mortem* but the cause of death could not be ascertained.

Histopathological examination of skin: Group I.—At 100 days (Fig. 2) the stratum corneum showed moderate to marked hyperkeratosis with occasional parakeratosis. In places there was mild infiltration with acute inflammatory cells, *viz.* mostly polymorphonuclear leucocytes and a few lymphocytes. The stratum Malpighii showed moderate to marked acanthosis with elongation of rete pegs, and a mild degree of spongiosis at a few places. The hair follicles mostly appeared normal except at a few places where the internal epithelial root sheaths appeared amorphous and stained bright red, and the external epithelial root sheaths showed some thickening. The hair shafts mostly appeared normal. The sebaceous glands showed metaplastic change in their cells which resembled squamous cells with vesicular nuclei. In the dermis the pars papillaris was deepened by elongation of the rete pegs and, along with the pars reticularis, showed congestion and occasional infiltration with polymorphonuclear leucocytes and lymphocytes. Fibroblastic activity was slightly increased in the dermis.

At 200 days the stratum corneum showed no hyperkeratosis or parakeratosis in 3 out of 4 animals killed. Only in one animal (25%) was mild to moderate degree of hyperkeratosis noticed. The rete Malpighii along with the stratum basale showed either no change, or mild to moderate acanthosis with occasional elongation of rete pegs. Hair follicles were seen to be markedly reduced in all the animals, constituting only 1–3 rows of sparsely placed follicles (normally there are 4–6 rows of closely placed hair follicles). Several of the existing follicles were seen to be empty with or without thickening of the external epithelial root sheaths. Hair shafts were very few and those existing were either short and truncated or appeared to be replaced by finely granular bluish-pink-staining material. At yet other sites whole shafts and follicles had disappeared leaving dermal papillae as remnants. Sebaceous glands also appeared to be reduced in number and at places only a few isolated groups of cells representing the remains of sebaceous glands were seen. In the dermis, the pars papillaris was seen to be normal in other animals except the one mouse which showed mild hyperkeratosis and acanthosis of the rete Malpighii. Here in this animal, rete pegs were elongated and at places showed vascular congestion and mild infiltration with inflammatory cells. The pars reticularis in all the animals showed a mild degree of fibroblastic activity with increased collagen content, the collagen fibres appeared thick and plump and their orientation was somewhat haphazard and disturbed. In the animal with hyperkeratotic and acanthotic changes, mild infiltration by polymorphs and lymphocytes was also noticed in the corium.

At 300 days (Fig. 3), the overall pattern was similar to that seen at 200 days. The loss of hair was even more marked. Where epidermal ulcers had healed there was a thin layer of young keratinized epithelium under which intense local infiltration with inflammatory cells was seen.

Histopathological examination of the skin: Group II.—At 100 days, the normal histological pattern of the different layers of skin was seen. Only one out of 5 animals (20%) showed mild parakeratosis in the stratum corneum, and a few inflammatory cells in the dermis. At 200 days, the stratum corneum showed no change or mild hyperkeratosis with occasional infiltration by polymorphonuclear leucocytes and lymphocytes. The rete Mal-

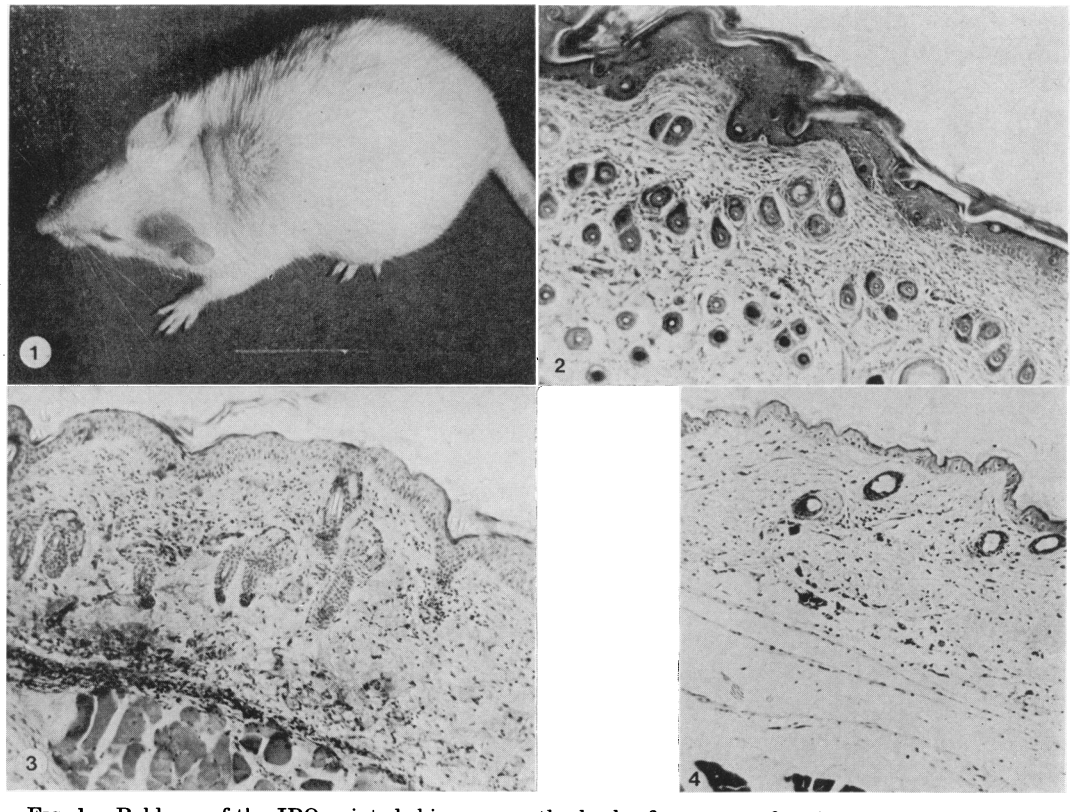


FIG. 1.—Baldness of the JBO-painted skin area on the back of a mouse after 21 weeks (146 days) painting.

FIG. 2.—Skin showing marked hyperkeratosis, acanthosis and mild dermal hyperplasia after painting with JBO for 100 days. H & E × 68.

FIG. 3.—Skin showing mild hyperkeratosis, acanthosis with degenerative changes and marked loss of hair appendages. Dermis shows mild hyperplasia. JBO painted for 300 days. H & E × 102.

FIG. 4.—Skin of mouse painted with acetone only for 300 days. Whole thickness of skin is reduced, along with reduction in number and density of hair appendages. Collagen fibres in dermis swollen, fragmented and sparse. H & E × 68.

pighii showed minimal to mild acanthosis and spongiosis. The dermis was sometimes slightly infiltrated with inflammatory cells both in the pars papillaris and the pars reticularis. No increase in fibroblasts or their activity, or in collagen tissue was seen. At 300 days, the changes observed were similar to those found at 200 days.

Phase II study

Gross examination.—None of the control (acetone-painted or Group A) animals developed tumours at any stage; however, animals from this group as well as from Groups B (50μg group) and C (5μg group) started showing poor hair growth in the painted area from 15 weeks onwards. Animals belonging to Group D (JBO + 5 μg BP) showed this change much earlier, *i.e.* after 8 weeks of painting only, which resulted in baldness of the painted area after 9 weeks. Acne formation and ulceration was noticed in Group D by about 18 weeks. Tumour development was first noted in Group B (50 μg BP) mice by 14 weeks when 6 out of 9 surviving mice (66·6%) had developed tumours on their backs. By 20 weeks when animals were killed, all (9/9) had developed tumours on the painted area. Mostly these tumours started as minute excrescences firmly attached to skin which kept on growing

into finger-like processes. In several animals more than one tumour grew at the same time. As they increased in size a few of these tumours sometimes coalesced, and as they grew in height they became more fragile. At no stage did these tumours become attached to deeper structures.

Animals belonging to Group C (5 μg BP) developed tumours after about 21 weeks of painting and those of Group D after about 17 weeks (*i.e.* 4 weeks earlier than Group C). The gross characters of tumour development were similar in these groups to those in Group B except in one animal of Group C, in which a pea-sized firm swelling inferior, postero-inferior, posterior and deep to the left ear, was also noticed at about the same time as a tumour in the painted area on the back was observed to be forming.

In all the remaining groups (Group A, C and D) except Group B (where only one animal died during the experiment), an unusual experience of gradual animal mortality was encountered which became noteworthy after 20 weeks of painting. It was more in experimental animals than the control (A) group. It was so marked an effect that by 43 weeks only one and 2 animals survived in Groups C and D respectively as compared to Group A, in which 4 animals had survived. However, by the end stage, tumours of skin from 4 mice of Group C and 3 mice from Group D were still available for histological study, as some of these mice had died after tumour development (Table II). Gross and microscopic examination of viscera (liver, spleen, kidney, heart, urinary bladder and stomach) from all animals which had died or been killed during the course of the experiment revealed no tumour development. Only local skin tumours in Group B, C and D were encountered in animals which survived long enough to develop tumour(s) or up to the terminal stage of experiment.

Microscopic examination of skin.—In Group A animals there was definite thinning of the whole thickness of the skin. In the epidermis there was mild to moderate hyperkeratosis with simultaneous thickening and thinning of the rete Malpighii at different places. Mild acanthosis was seen at places of thickening with loss of prickles. The stratum basale at a few places was well defined, while at thinned-out areas it was not distinguishable from the upper layers, which were only 2–3 cells thick. The hair shafts and follicles appeared normal although much decreased both in number and row formation (only 1–3 rows seen). The dermis, apart from appearing much reduced in thickness, showed swelling and fragmentation of collagen fibres which were otherwise loosely arranged with plenty of interstitial space between them. A mild degree of vascular congestion was also noted in the dermal area (Fig. 4).

In Group B and C there was essentially no difference in the character of tumour formation. Tumours ranged from papillomatous keratoacanthomata with isolated areas of low-malignancy squamous-cell carcinoma to frank squamous-cell carcin-

TABLE II.—*Induction of Skin Tumours by Painting 3,4 Benzpyrene (Dissolved in Acetone) with Jute-batching Oil (Phase II Study)*

	Number of mice developing skin tumours (weeks after start of painting)									Total number of mice developing tumours*	Average induction time of first tumour (in weeks)
	5	10	15	20	25	30	35	40	43		
A. Control†	0/10	0/9	0/8	0/8	0/5	0/5	0/5	0/4	0/4	—	—
B. 50 μg BP Group	0/10	0/10	6/9	9/9	Killed after 20 weeks					9	14
C. 5 μg BP Group	0/10	0/10	0/10	0/10	1/7	1/5	2/3	1/1	1/1	4	21
D. 5 μg BP+JBO Group	0/10	0/10	0/10	1/9	2/7	2/6	2/5	2/3	2/2	3	17

* Results should be interpreted with caution due to high mortality of mice in all groups carried ahead after 20 weeks (see text).

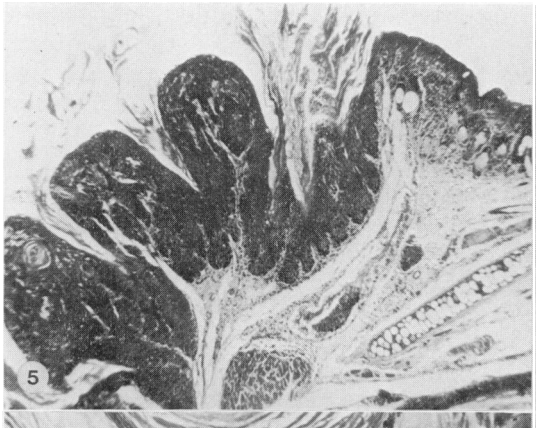
† Painted with acetone alone thrice weekly.

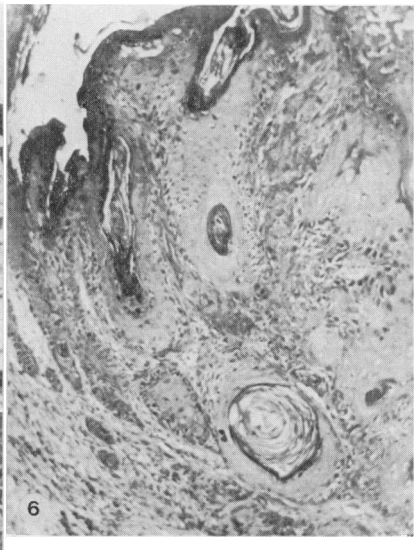
omata with varying degrees of keratin pearl formation. Some of these carcinomas were accompanied by basal cell proliferation and/or features of basal-cell carcinoma type as well (Fig. 5). In a few of these tumours infiltration with acute inflammatory cells such as polymorphonuclear leucocytes or even abscess formation was seen. In one Group C animal in which there was a swelling near the left ear, this was found to be due to basal-cell carcinoma which had extended deeply up to the vicinity of the parotid gland but did not involve it. Those tumours were friable on gross examination and contained large amounts of keratin.

Animals belonging to Group D developed similar varieties of skin tumour to those in Groups B and C but with a few additional features. Dermal collagen fibres were increased, with plump and dividing fibroblasts; infiltration with macrophages was noticed in the dermis, and at a few places sheets of epithelioid cells in the dermal zone were seen, their nuclei and cytoplasmic characteristics being very similar to those of macrophages. Infiltration with polymorphs, lymphocytes and plasma cells was also seen in the corium (Fig. 6).

DISCUSSION

In the first phase of the study, in which JBO alone was applied thrice weekly,





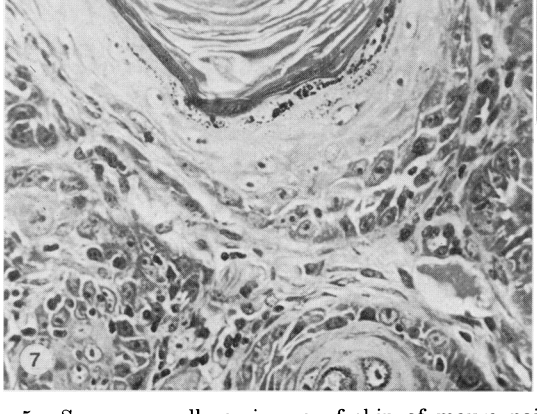


FIG. 5.—Squamous-cell carcinoma of skin of mouse painted with 3,4 benzpyrene. Keratin pearl formation and basaloid features seen. H & E × 75.

FIG. 6.—Squamous-cell carcinoma with basaloid features and keratin pearl formation. Also dermal hyperplasia and macrophage infiltration seen in the skin of mouse painted with BP + JBO. H & E × 75.

FIG. 7.—Neoplastic proliferation of squamous as well as basal cells of epidermis with keratin pearl formation in skin of mouse painted with BP + JBO. H & E × 300.

mice showed poor hair growth after 18 weeks which progressed to baldness after 21 weeks, by which time acne and small ulcers developed in 12·5% (one out of 8) animals, this figure rising to 50% (4 out of 8) animals by 25 weeks. At no stage did the control group show any of these changes. In neither group was tumour development noticed up to the end of the experiment (300 days). On microscopy first carried out at 100 days in the JBO-painted mice, moderate to marked hyperkeratosis and parakeratosis in the stratum corneum, along with acanthosis and spongiosis in the Malpighian layer, and elongation of rete pegs, was noticed in the epidermis. There was some evidence of metaplastic change in sebaceous glands cells, many of which had undergone transformation into squamous cells, with vesicular nuclei. The corium showed a mild increase in fibroblastic activity with slight congestion and infiltration by polymorphs and lymphocytes. Hair follicles and hair shafts remained more or less unaffected. However, when skins of JBO-painted animals were examined after 200 and 300 days, the hyperkeratosis and parakeratosis were found to have practically disappeared and the rete Malpighii showed either no change at all, or mild to moderate acanthosis in 75% animals. It appeared that the skin had become tolerant to the initial irritating effect, but the major effect was observed in the whole hair apparatus, where both hairshaft and follicle were affected by degenerative changes; many of those had finally disappeared altogether, being represented by residual dermal papillae. A similar fate attended the sebaceous glands. The ulcers and acne which had developed were also found ultimately to heal, gaps being bridged by a thin layer of newly-formed keratinized epithelium.

As no gross or microscopic indications of neoplastic changes were noticed after JBO painting of ITRC mice (*cf.* the earlier report of Roe *et al.* (1967), who found their JBO sample to be carcinogenic), the question arose whether the mice chosen for study were cancer-resistant. To eliminate this possibility and (since JBO was not found to be carcinogenic in our Phase-I study) to establish whether it was a cancer-promoting agent, the Phase II study employing the same strain of mice and BP as carcinogen was planned, along with UV and IR spectroscopy of the JBO sample to see if it contained any traces of polycyclic aromatic hydrocarbons.

In the Phase II study, pattern of painting, vehicle for BP and techniques adopted closely followed those of Lalitha Kumari, Sirsi and Bhargava (1974). It was found that mice painted with 50 μg of BP thrice weekly developed tumours confined to skin after 14 weeks, by 15 weeks 6/9 (66·6%) animals belonging to this group had developed tumours, and by 20 weeks 9/9 (100%) animals developed skin tumours confined to the painted area. These tumours were initially papillomatous in nature but soon malignant changes also occurred in these and adjoining areas. Several of these tumours also had basaloid features. Keratin was usually produced in huge quantities, making the tumours friable. The group painted with 5 μg of BP alone per painting started to get skin tumours after 21 weeks but the combined group (*i.e.* mice painted simultaneously with 5 μg of BP and JBO) developed tumours by 17 weeks (4 weeks earlier than the 5μg BP alone group). The nature of tumours in both these groups was similar to that found with the 50μg BP group. The fact that spectroscopically no traces of PAH were found in the JBO sample, and yet the combination of both JBO and BP reduced the latent period for tumour development (as compared to the equivalent BP-painted group), suggests that JBO may be a cancer-promoting agent. On the other hand this activity may be entirely due to a nonspecific irritating effect which favoured the earlier tumour growth in presence of BP, a standard tumorigenic agent.

The finding that mice belonging to both control (application of the BP vehicle, acetone, only) and experimental groups

faced an unusually high rate of mortality, especially after 20 weeks of painting, suggested that probably the quantity of acetone employed per painting, *i.e.* 0·1 ml thrice weekly, was toxic to the animals. It would therefore seem that in future studies these mice should not be painted with a quantity of acetone exceeding 0·05 ml per application. It would also seem that the acetone itself, when applied alone, brought about thinning of the whole skin thickness with damage to and degeneration of collagen in the corium and the disappearance of whole hair apparatus leading to sparse hair growth over the painted area. This effect of acetone combined with that of JBO was in essence responsible for the earlier appearance of baldness, acne and ulceration in the combined group in our Phase II study. In this group, apart from tumour development, on histological examination hyperplasia of dermal collagen tissue, macrophage infiltration and attempts at epithelioid-cell formation were seen in the dermis at 300 days. Increased fibroblastic activity and the laying down of collagen tissue was also seen in mice painted with JBO alone in our Phase I study by this time, but other changes such as macrophage infiltration and epithelioid cell formation were peculiar only to the combined group of the Phase II study; the precise pathogenesis of this remains unexplained.

It has thus been found that the jute-batching oil sample tested on mouse skin under the conditions described above was non-carcinogenic. Although initially the mice seemed to have suffered irritating effects from the jute-batching oil, resulting in hyper- and parakeratosis, poor hair growth, baldness, acne and ulceration over the painted area, subsequently they at least partially adapted themselves to this effect. Only the baldness and loss of hair appendages along with hyperplasia of the dermis persisted, while acne and ulcers healed in spite of continued regular painting with jute-batching oil in the same quantities.

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