

IV REPORT ON LOKSTEDT SKIN-PAINTING EXPERIMENTS
COMMENTS ON TRC PAPER K539

The long-term results are examined in various ways and corrections made for differences in the spreading of the various condensates. In this brief note the standardised numbers of tumours (Table 6 in paper K539), before correction for spreading, are compared in Table 1. The data is based on tumours confirmed by histology at the end of the experiment and regressions are excluded.

The results may be summarised as follows:

1. Gerlach Sheet is very much less active than the control and the result is not dependent upon sheet density.
2. Schweitzer Sheet is much less active than the control.
3. AMF Sheet is comparable with the control.
4. Arenco and Borgwaldt Sheets are both more tumorigenic than the control, but the difference for Arenco is small.
5. Nitrate added to tobacco produces a large reduction in activity; activity is also reduced when nitrate is added to Gerlach sheet.

Table 1

| Code | Sample | Tumours Standardised (SH) | | Hyperplasia Area Activity | |
|------|-------------------|---------------------------|--------|---------------------------|-----------------|
| | | Dose 1 | Dose 2 | 1967 | 1970 |
| E2 | Control tobacco | 28 | 29 | 83 ¹ | 92 |
| EA | Arenco sheet | 30 | 34 | | 90 |
| EB | Borgwaldt sheet | 39 | 31 | | 102 |
| EG1 | Gerlach sheet: { | 70-80 g/m ² | 8 | 10 | 62 ² |
| EG2 | | 36-42 g/m ² | 8 | 9 | 44 ³ |
| EG3 | | 8% nitrate | 3.4 | 3.4 | 40 ⁴ |
| ES | Schweitzer sheet | 15 | 12 | | 88 |
| EW | AMF sheet | 26 | 32 | | 107 |
| EN | Tobacco + nitrate | 8 | 8 | | |

Samples based on earlier blend E: Codes 1 = Blend E, 2 = Folie E1, 3 = Folie E2, 4 = Folie E3

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Comparison with Hyperplasia Tests

The results obtained on samples based on an earlier manufacture, blend E, are lower than those based on sample E2. The 1967 activity ratings predicted the low results for Gerlach sheet, including a small reduction with added nitrate, but incorrectly suggested a significant effect due to sheet density.

The values obtained in 1970 (BTC document 200/4) indicated the significantly reduced activity for Gerlach sheet and the intermediate position of Schweitzer sheet. Although the ranking of the Schweitzer sample was correct, a significant difference was not found in the short-term test. The hyperplasia test also predicted that the Arenco sheet was similar in activity to the control. High values were predicted for the Borgwaldt sheet (correct) and also for the AMF sheet; the latter prediction was incorrect since in the long-term test the activity of AMF was comparable with the control.

The rankings, with indications of significant differences marked (\ll) are illustrated:

Long-term:

Gerlach
+ \ll Gerlach \ll Schweitzer \ll Control
Nitrate

and Control = AMF \doteq Arenco \ll Borgwaldt

Hyperplasia:

Gerlach
+ \ll Gerlach \ll Schweitzer \leq Arenco \leq Control
Nitrate

and Control \ll Borgwaldt \ll AMF.

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INTRODUCTION

It is feasible that individual fractions of cigarette smoke could contain both carcinogenic and irritant substances. The specific tissue reaction produced when compounds are present separately has been identified from our earlier work but no experience is available in interpreting the tissue reaction produced when both carcinogens and irritants are present together. Accordingly a series of model experiments was devised in which various combinations of chemicals whose irritant and carcinogenic potentials were known, were injected subcutaneously in rats and the resulting tissue response analysed as to its usefulness in reflecting the properties present in such combinations.

EXPERIMENTAL

Model situations were envisaged to give four combinations of activities. Activity in this sense is defined as the ability of a compound at a given concentration to produce a characteristic lesion in the subcutaneous tissue on short term tests and to produce local sarcomas on long term testing

Group 1 Carcinogen and irritant both active

Group 2 Carcinogen active, irritant inactive

Group 3 Carcinogen inactive, irritant active

Group 4 Carcinogen and irritant both inactive

The carcinogen used was benzpyrene and the irritants were Tween 80, and ethanol/tricaprylin solutions. From previous work in the preliminary stages of this project, it was known that 1 µg benzpyrene produced no changes in the tissue at the injection site indicative of a carcinogen but 100 µg benzpyrene produced on injection the characteristic lesion elicited by a direct acting carcinogen (Hooson & Grasso 1971). Similarly experiments had shown that 1% aqueous solutions of Tween 80 had no

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irritant action on the subcutaneous tissue (although raising the concentration to 6% with consequent increase in surface activity, restored irritant properties). 1:1 alcohol/tricaprylin mixtures were found to produce a characteristic irritant lesion in the subcutaneous tissue. Both low concentrations of Tween 80 and alcohol/tricaprylin mixtures will act as solvents for benzpyrene, therefore the four model groups were set up as follows.

- Group 1 100 µg benzpyrene dissolved in 1:1 alc./tricaprylin
- Group 2 100 µg benzpyrene in 1% Tween 80
- Group 3 1 µg benzpyrene in 1:1 alc./tricaprylin
- Group 4 1 µg benzpyrene in 1% Tween 80

Twenty rats were used in each experiment and injections of the above solutions were given twice weekly into the same subcutaneous site. One male and one female animal were killed 24 hrs after each injection and the injection site excised, fixed processed and stained in the usual manner.

RESULTS

When carcinogen and irritant were both present in active concentrations, an acute lesion was manifest at the site in the early stages but granulation tissue appeared after the first week and a heavy collagen deposit accumulated. No indication of an inhibition in the process of connective tissue repair was seen. In contrast, animals in Group 2 exhibited a delay in granulation tissue formation at the site of injection. When fibroblasts did eventually appear, about 10% were enlarged, misshapen, and showed nuclear abnormalities. Deposition of young, newly formed collagen was scanty and collagen already present had undergone a fibrinoid degeneration. Injection site in rats from Group 3 showed the same acute lesion and the subsequent progression of histological response that has been described

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previously for Group 1. As observed in this set of animals, sites taken in the later stages of the experiment were heavily collagenised and foci of dividing fibroblasts could be detected in certain areas. An essentially unchanged connective tissue architecture was recorded at the injection site of rats in the last group, although occasionally small fibrin cysts, marking the site of injection 24 hrs previously, were present in the subpannicular layer. Accompanying these cysts was a variable infiltrate of polymorphs, lymphocytes and histiocytes.

DISCUSSION

Compounds which possess certain physico-chemical properties capable of acting as irritants and producing tissue injury have been found on repeated injection over 5 weeks to give a characteristic lesion at the site. (Grasso and Golberg 1966a). The reaction is marked by an extensive build up of collagen after an initially severe acute inflammation.

This aberrant connective tissue repair can, in time, progress to neoplasia, malignant change occurring in the fibroblasts that continue to divide amongst the thick collagen bands. (Hooson & Grasso 1971 - unpublished).

In contrast, direct carcinogens produce a different histological picture at the site of their injection. The normal process of connective tissue repair is delayed, granulation tissue, when it finally forms is irregular and the constituent fibroblasts exhibit morphological abnormalities.

The preceding experiments were designed to see to what extent these histological processes remained discrete when chemicals capable of producing both effects were injected together. The results obtained show that when both compounds are present at active concentrations, the carcinogenic reaction is totally masked by the tissue response to the irritant. The results also demonstrate that a tissue responding

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to either a carcinogen or an irritant has no altered sensitivity due to the presence of the other compound in normally inactive concentrations. Also when both compounds are present at inactive concentrations no synergism occurs.

When these findings are extrapolated to the problem of the tissue reaction elicited by various fractions of smoke, several interesting facts emerge. It is known that direct carcinogens are present in certain fractions, but at concentrations unlikely to produce a carcinogenic effect per se. From the above results, it does not seem that these low doses of carcinogen would enhance the action of the irritants that are known to be present in the fractions. However, the results also show that a chemical constituent of the fraction acting as a direct carcinogen would not be detected in this test if an irritant chemical was present in the same fraction. This does not however invalidate the use of the subcutaneous route as a means of comparing the irritancy of the various fractions. If irritation is the basic cause of the tumorigenicity of cigarette smokes, identification of the responsible chemicals is an essential first step to their elimination.

Synopsis

On the basis of the above experimental results, it appears that the simultaneous presence of carcinogens and irritants cannot be detected by injection of both compounds simultaneously. The irritant action of one compound will mask the carcinogenic type of reaction provoked by the second compound.

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References

Grasso, P. & Golberg, L. (1966). Early changes at the site of repeated injections of food colourings.

Fd Cosmet. Toxicol. 4, 269.

Hooson, J. & Grasso, P. (1971). Early reactions of the subcutaneous tissue to repeated injections of carcinogens in aqueous solutions.

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CAFFEINEInhibitory Effect on Carcinogenicity of Condensate Fractions

The effect of adding caffeine to smoke condensate fractions has been examined at Harrogate. Caffeine was added at two dose levels (0.04% and 0.2% of whole smoke condensate) to fraction G (cyclohexane soluble, water insoluble), and to fraction (R + P)G (Formic acid soluble + caffeine complex from fraction G) of condensate produced from a 'standard' flue-cured cigarette.

The results show that :

- (1) 0.2% caffeine (whole smoke equivalent) reduces the activity of the fractions by about 40% : the tumorigenic ratios were 0.60 for tumour bearing animals and 0.63 in respect of infiltrating carcinomas.
- (2) With a smaller dose of caffeine, 0.04% of the whole smoke equivalent, a small reduction (ca 10-15%) in activity was observed, but this was not statistically significant; tumorigenic ratio 0.85 and, for infiltrating carcinomas, 0.89.

Assuming that it was desired to capitalise on this finding and to investigate the effect of 0.2% caffeine on whole smoke condensate, as distinct from fractions, then it would be necessary to add caffeine, so that a cigarette yielding 20 mg TPM also produced 0.4 mg caffeine in the condensate. Work in GR & DC has shown that the transfer of caffeine is about 18%, thus the amount of caffeine added to the cigarette would have to be approximately 2.2 mg.

If it is considered that further investigation is warranted, then it is suggested that the initial work be limited to the production of sufficient cigarettes for assessment in short-term tests and by smoking panels. Depending on satisfactory results, promotion or long-term carcinogenicity studies could then be initiated.

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