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PROJECT JANUS *Aut*

ANNUAL REPORT 1969-70

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PROJECT JANUS
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The major portion of the work on Project JANUS at the Battelle Institut, Frankfurt, continues to be concerned with long-term mouse-skin painting experiments. All the mice in the first three experiments are now dead and almost all the detailed histological examination of the sections is complete.

The data from the first experiment (B0) have been coded and transferred to the computer at Millbank. Although the information required on tumour bearing animals has been obtained, and a preliminary statistical evaluation undertaken, other aspects of the scheme have still to be checked.

An analyst/programmer from an outside agency has been engaged so that the computer system can be updated in the light of experience. When this has been undertaken, the results of the other completed experiments will be data-coded and transferred to the computer store for subsequent recovery and analysis.

The Hyperplasia Test is being used for the short-term evaluation of cigarette samples on a routine basis. Detailed plans have been formulated to examine the promoting activity of smoke condensate. A twenty-six week feasibility study will be started later in 1970.

During the year there have been further staff changes at Battelle. Following the departure of Dr. Hofmann, Dr. Kiendl took over responsibility for the JANUS programme under Dr. F.A. Sacherer, who was appointed as head of the toxicology section. Dr. Karbe, a pathologist, has recently joined this section but a successor has

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not been appointed to replace Dr. Eder, who was head of the biology department.

LONG-TERM TEST

It is emphasised that the results from the following experiments have not been analysed fully and the following comments are based on a preliminary evaluation of the age-standardised results.

Experiment B0 (150 weeks, all mice dead)

The initial experiment, in which smoke condensate from a flue-cured lamina blend was examined, was designed to ensure that a long-term test could be undertaken successfully at Battelle. At the same time, it was hoped that the tumorigenic response would provide a reference point for comparison with the results from other long-term tests carried out under Project JANUS.

The high tumour response and long life of the mice maintained under barrier conditions at Battelle implies that we have a sensitive test procedure.

It has become apparent, however, that this sensitivity has its own drawback in that the doses of condensate applied have been too high. Thus it is probable that it will not be possible to establish, from the initial experiments, a satisfactory dose-response

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curve which is essential for some of the more detailed statistical analyses.

Experiment B1 (136 weeks, all mice dead)

In this experiment, the effect of various puff volumes (10, 25 and 50 ml) on the tumorigenicity of the condensate was examined. The cigarette used was identical with that in Experiment B0 which was smoked using a puff volume of 35 ml.

The results of this experiment indicate that the activity of the condensate is higher at low puff volumes.

Experiment B2 (160 weeks, all mice dead)

The smoke condensate from a "typical" US KSFT cigarette was examined. The results indicate that the activity of the condensate is intermediate between that obtained from flue-cured lamina (B0) and that from cigarette B4 (see below). These differences may not be statistically significant.

Experiment B3 (152 weeks, all mice dead)

The cigarette examined was manufactured from 100% PCL produced in Montreal. The PCL flour was prepared from CN102 lamina and the binder from Canadian flue-cured stem.

It is clear from the preliminary evaluation that the activity of this condensate is considerably less than that from flue-cured lamina; it is also lower than that from the cigarette made with equal proportions of flue-cured lamina and Canadian flue-cured stem (Experiment B4). It is probable that the low activity of the condensate will be statistically significant.

Experiment B4 (140 weeks, in progress)

The cigarettes were manufactured from equal proportions of

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flue-cured lamina and Canadian flue-cured stem (as CRS). Only a few mice, all treated at the lowest level, are still alive.

The preliminary age-standardised results indicate that the incorporation of CRS at the 50% level leads to an appreciable reduction in the specific activity of the condensate (Experiment B4 vs. B0). The comparison with Experiment B3 has been noted above.

Experiment B5 (140 weeks, in progress)

As there is no guarantee that the response of different batches of mice is the same, the condensates used in Experiments B0, B2 and B3 are being re-examined in this experiment. There is no indication that a large change in the response of the mice has occurred.

The experiment is nearly complete and it is encouraging that the age-standardised results are very similar to those obtained previously.

A second factor being examined is the effect of changing the puffing frequency from once per minute to three times per minute. The current results indicate that this change in puffing frequency has little, if any, effect on the specific activity of the condensate.

Experiments B6 & B7 (96 weeks, in progress)

These experiments were combined to examine the following effects:

- (a) Different strand widths (30, 60 and 120 cpi) using two types of tobacco (B7 control, and B6).
- (b) Yeast-treatment of flue-cured tobacco (B6).

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At the present time it appears that the effect of strand width is marginal and it is unlikely that there will be a clear indication of a preferred strand width. Earlier, it was thought that yeast treatment was giving rise to a condensate with a reduced activity. This misleading indication was largely a result of the toxicity of the condensate which led to an abnormally high death rate in the early weeks of the experiment. Corrections made by the age-standardisation technique now suggest that yeast-treated tobacco is unlikely to lead to a condensate with reduced tumorigenicity.

Experiment B8 (40 weeks, in progress)

As outlined in last year's report, six samples are being used to examine the effect of PCL (X-PCL-5) ex Louisville and a Celanese smoking material (I-308). A cigarette with a silica gel filter is also included. The samples are:

1. Control tobacco, flue-cured lamina (50%) and CRS (50%).
2. X-PCL-5 (60%) and control tobacco
3. X-PCL-5 (100%)
4. I-308 (60%) and control tobacco
5. I-308 (100%)
6. Control cigarette with a silica gel filter

Only a small number of tumours have appeared in most cases and it is far too early to draw conclusions. It is of interest, however, that the predicted activity of the condensate from the sample with the silica gel filter is considerably higher than that from the control tobacco.

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Experiment B9 (4 weeks, in progress)

In this experiment six samples are being used to examine the effect of ethanol extraction, and of various aspects of Gerlach tobacco reconstitution process. The samples are:

1. Control, flue-cured lamina (60%) and Canadian flue-cured stem (40%). (Approximates to B4).
2. Tobacco extracted with ethanol.
3. Tobacco extracted with ethanol, extract returned.
4. PCL manufactured from control blend with Gerlach additives.
5. Gerlach sheet material manufactured from control tobacco.
6. Gerlach sheet manufactured from ethanol-extracted tobacco.

The results from this examination should provide a direct comparison of PCL and Gerlach sheet and also indicate whether it is possible to combine the advantages reported for both ethanol extraction and the Gerlach process.

The start of this experiment was delayed for some months owing to the inability of ASL Ltd. to supply mice of a standard sufficiently high for long-term experiments. As a result, mice are now being obtained from Carworth (Europe); this company already supplies mice to TRC, Harrogate and the Huntingdon Research Centre.

General Report

A report giving details which are generally applicable to the JANUS long-term experiments has been issued.

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HYPERPLASIA TEST

During the year, more than fifty cigarettes were examined, including samples from Montreal, Louisville and Sydney. The number of samples examined is higher than expected because additional tests became available due to the delay in starting Experiment B9. A considerable number of samples were manufactured from reconstituted tobaccos. The importance of the process was again noted and, although the anticipated reductions in activity were found for PCL, Gerlach and Schweitzer materials, comparable results were not obtained for Borgwaldt or Philip Morris materials. Significant reductions were noted with PRT material produced in Southampton. The samples for Experiment B9 have been tested using ASL and Carworth mice, although the latter results are not yet available. The initial results indicate that the reduction obtained with the PCL sample is comparable with that for Gerlach and also suggest that the effect of ethanol extraction and sheet manufacture may be additive. The results would be of considerable importance if they are confirmed in the long-term test.

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OUTLINE 1970-71

GENERAL

During the year the contract with Battelle Institut was renewed for a further five years.

The long-term mouse-skin test is likely to remain as a major test for some years. In various laboratories a great deal of effort is being expended on various inhalation tests. So far these have been unsuccessful although studies, such as that undertaken by Auerbach using beagle dogs, have received much publicity. It is likely that it will still be some time before inhalation techniques, capable of comparing the tumorigenicities of different cigarettes, will be available.

It is planned to continue to use the short-term Hyperplasia test on a routine basis. Although it is not a substitute for long-term tests and anomalous results must be anticipated, it is of considerable assistance in guiding experimental work in various laboratories.

Interesting short-term assays such as the sebaceous gland test and mouse irritancy screen are being used at Huntingdon as well as the sebaceous gland test at Hamburg. A number of cigarette samples used in the JANUS programme have been submitted for testing at these centres. Conversely, samples from the Lokstedt programme which have been tested at Hamburg are currently being examined at Huntingdon and, using the Hyperplasia test, at Frankfurt. At the present time it would seem more profitable to intercompare samples on this basis rather than attempting to duplicate the tests at Battelle.

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Since it appears that the action of carcinogenic materials may be accelerated by so-called promoters, a feasibility study to examine the action of smoke condensate as a promoter has been planned. If successful, it is hoped to examine various cigarette samples using this as a medium-term (26 week) test.

LONG-TERM TESTS

With the continuing interest in various reconstituted tobacco materials, the mouse-skin painting facilities will be used for further comparisons of this nature. Experiment B9, which has only just started, has already been outlined.

In a further experiment (B10), it is planned to compare PCL manufactured in Canada and Schweitzer sheet from P.J. Schweitzer in the United States. Although these materials have been tested previously, a direct comparison in a single experiment has not been undertaken. It was hoped to include additional samples in the same experiment but the use of a different strain of animals makes it even more important to attempt to establish a dose-response curve which can be used to intercompare the results of future experiments. For this reason, three dose levels will be used with each of the above samples and five dose levels will be employed for the control cigarette condensate. It should be noted that, following the

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observations on dose levels made at Southampton, discussions were held with other workers and these have led to recommendations for the use of lower dose levels at Harrogate and at Huntingdon.

It is expected that a second long-term experiment will be commenced during the summer of 1971. In this, it is hoped to examine the effect of a number of additives, and that these will include both conventional additives and chemicals designed to modify the combustion process. Following the reduction of activity noted as a result of ethanol extraction, it has been suggested that the effect is largely due to the extraction of almost all the sugars. It appears important to determine to what extent the reduction in activity is related solely to the sugars and that this could best be achieved by adding sugar to tobacco with a low natural sugar level. Although a number of chemical additives have been shown to lead to appreciable reductions in the level of aromatic polycyclic hydrocarbons, these have not proved successful when tested using the Hyperplasia test. Nevertheless, since this test does not necessarily parallel the long-term evaluation, it would appear worthwhile to include one or two such additives in a long-term experiment.

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HYPERPLASIA TEST

The results of some of the additional tests undertaken in the early summer are still awaited. Further samples have been prepared for testing in December and January; these include cigarettes manufactured at different circumferences, oval cigarettes and a series manufactured from PCL material made at different thicknesses.

PROMOTER TEST (Feasibility Study)

This study will start late in 1970, using new facilities constructed in the Biological Department at Battelle's expense. In this experiment the mice will be treated, at two dose levels, with the initiator, dimethylbenzanthracene. Subsequently condensate from two cigarettes will be applied at one dose level. The control groups will be painted with acetone. The experiment will be limited to about twenty-six weeks and no detailed histology will be undertaken. For the feasibility study, it has been decided to examine two samples currently being used in the B9 experiment, (B9-1 and B9-6). This choice has been made on the basis of the anticipated reduction in tumorigenicity of the ethanol extracted tobacco made into Gerlach sheet (B9-6). It should also be possible to make direct comparisons with the long-term test since Carworth mice will be used in both instances.

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REPORTS

| <u>Title</u> | <u>Report Number</u> |
|---------------------------------|----------------------|
| General Report on Project JANUS | B18 |

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