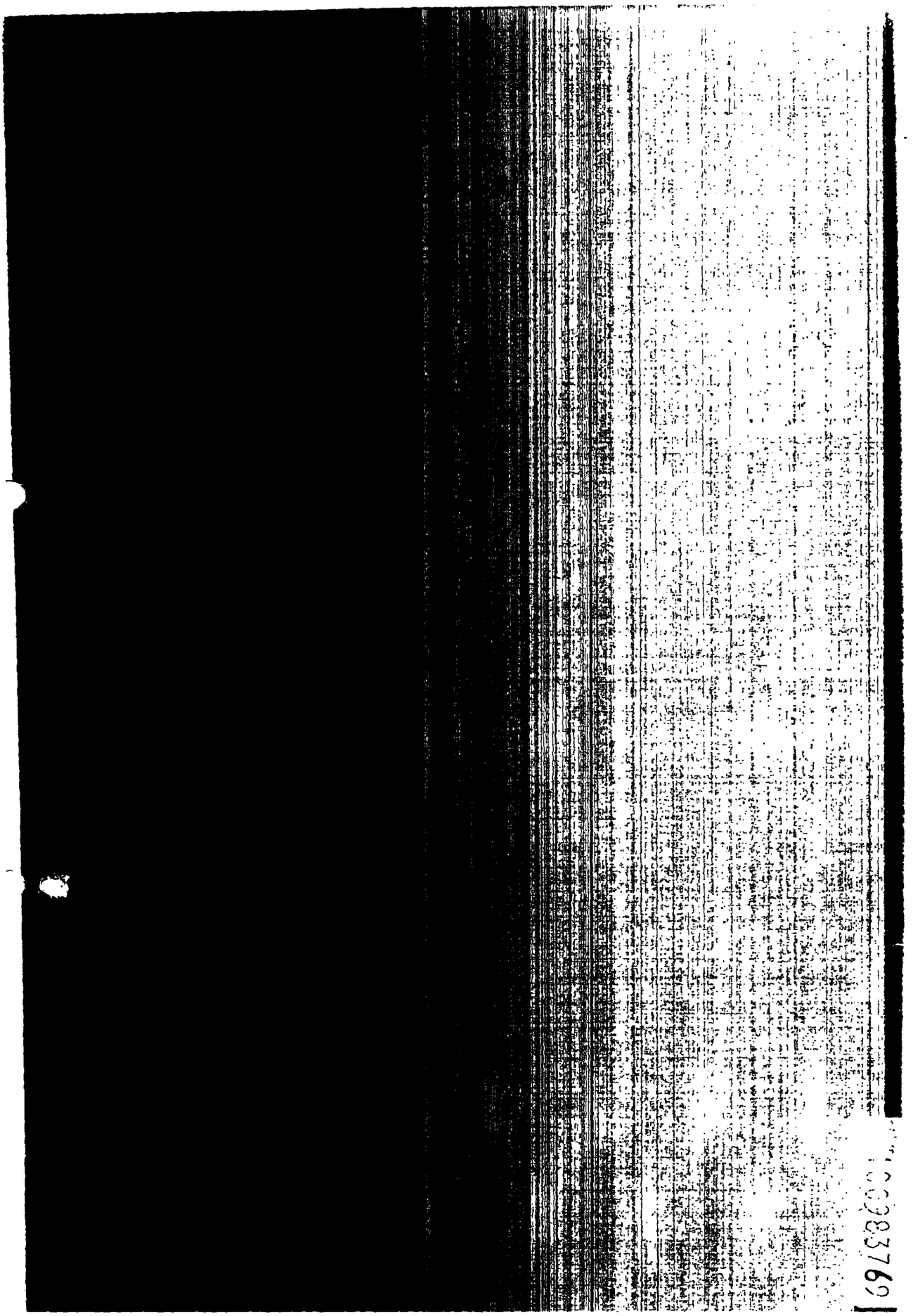

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BRITISH-AMERICAN TOBACCO COMPANY LTD.

PROJECT JANUS

ANNUAL REPORT

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AUTHOR: C.I. Ayres

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PROJECT JANUS

ANNUAL REPORT 1968/69

As with the previous years, the major portion of the work on Project JANUS at the Battelle Institut, Frankfurt-am-Main, is concerned with the examination of smoke condensate by a series of long-term skin-painting experiments. All the mice in the first experiment are now dead and the detailed histological examination of the sections will be finished in the next few weeks so that the complete data should be available for statistical analysis by the end of 1969.

Samples of cigarettes continue to be examined on a routine basis by the Hyperplasia Test, but the use of the Ciliastasis Test was terminated in March.

Mid-way through the year, Dr. Miedreich, the leader of the Project JANUS team at Battelle, left Frankfurt. His departure necessitated a degree of re-organisation of the staff at Battelle. Dr. Hofmann took over as leader of the team and has overall responsibility for the Project. Dr. Kiendl is responsible for the skin-painting programme (long-term tests) and Dr. Kramer joined the team to assist Dr. Konigsmann with the pathology. Dr. Militzer continues to operate the Hyperplasia Test.

LONG-TERM TESTS

Each experiment takes approximately 2½ to 3 years to be completed and an indication is given below of the main findings to date, together with the length of time the experiment has been in progress.

Experiment B0 (152 weeks) - All mice dead

The smoke condensate from a flue-cured lamina (CN102) blend was examined. The major, but perhaps simple, aim of this first experiment was to demonstrate that a long-term test could be carried out successfully at Battelle. At the same time, however, the tumorigenic response to this condensate provides a useful "bench-mark" or reference point for comparison with the results from other long-term tests carried out under Project JANUS, and, to a degree, for comparison with experimental results obtained elsewhere.

The high tumour response and the long life expectancy of the mice in the Battelle experiments means that we have a sensitive test procedure compared, for example, with T.R.C. Harrogate.

Experiment B1 (136 weeks) - All mice dead

In this experiment, the cigarette used was identical with that in the first experiment. Whereas in Experiment B0, a puff-volume of 35 ml was used to smoke the cigarette, in Experiment B1 a range of puff-volumes (10, 25 and 50 ml) was used.

The indication from the results, which have recently been confirmed by a T.R.C. experiment at Harrogate, is that the larger the puff-volume, the lower is the activity of the condensate.

Experiment B2 (140 weeks) - In progress

This experiment is an examination of a "typical" U.S. K.S.F.T. cigarette. The current indication from the results is that the activity of the condensate

is similar, but probably less than that from the cigarette containing flue-cured lamina.

Experiment B3 (128 weeks) - In progress

A cigarette made from 100% PCL is being examined in this experiment. The PCL was made in Montreal: the flour was prepared from CN102 lamina and the binder from Canadian stem.

It is clear from the current results that the activity of this condensate is less than that from flue-cured lamina. This is an encouraging and important finding.

Experiment B4 (92 weeks) - In progress

The cigarette for this experiment was made from equal portions of flue-cured lamina (CN102 blend) and Canadian stem (as CRS). As an approximation, therefore, it contains the "starting tobaccos" in the proportions which are used to made PCL.

The current results indicate two important findings:

(a) Increasing the level of CRS leads to a reduction in the specific activity of the condensate (Experiment B4 vs. Experiment B0).

(b) The processing of tobacco into PCL leads to a reduction in the specific activity of the condensate (Experiment B3 vs. Experiment B4).

Experiment B5 (92 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.

A second point 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 and B7 (48 weeks) - In progress

Two experiments were combined so that the effects could be examined of:

(a) A change in strand width (30, 60 and 120 cpi) using two types of tobacco.

(b) Yeast treatment of flue-cured tobacco. The process was based upon the earlier work under Project AIRFERM.

Whilst it is too early in the experiments to draw firm conclusions, and it must be emphasised that the picture could change in the next few months, the initial results indicate that:

(i) The effect of a change in strand width is such that a minimum activity is associated with 60 cpi. The differences, however, are only small.

(ii) Yeast treatment might lead to a small reduction in the specific activity of the condensate.

HYPERPLASIA TEST

During the year, over forty samples of cigarettes were examined by the Hyperplasia Test. Several of these were semi-commercial samples examined at the request of Montreal, Louisville and Hamburg. The coating of cigarette paper with aluminium was shown to have no effect on the response in the Hyperplasia Test. As part of a joint exercise with Harrogate and Battelle, Geneva, a series of eight T.R.C. samples were examined. These samples involved changes in circumference, cpi and paper porosity, but no clear trends were apparent in the Hyperplasia results.

A series of twelve samples of cigarettes containing PCL or Batex made from different starting tobaccos was examined. An important inference from the results was that not only does the process affect the activity of the condensate but the nature of the starting tobaccos is also of significance.

CILIASTASIS TESTS

Before the work was terminated in March, Battelle had examined 24 samples of cigarettes by the Clam Gill and Rabbit Trachea tests. In no case, however, was a low toxicity smoke observed. This, perhaps, was largely to be expected since the majority of the samples were plain cigarettes which had been examined by the Hyperplasia test and their evaluation by the Ciliastasis tests was undertaken mainly to complete the biological assay. Even the few filter-tipped samples (e.g. bonded polyurethane) examined were not expected to have a low smoke toxicity but were examined as a safeguard against missing the "unforeseen" result.

MAST CELLS

There is a claim in the literature that, prior to the appearance of a papilloma, there is an increase in the number of mast cells in the epidermis of a mouse. It was possible, therefore, that a monitor of mast cell concentration might form the basis of a useful short-term test. Battelle examined a number of skin-sections from the mice which had died during the long-term Experiment BO, but the results were not encouraging and the approach was terminated.

FREI AND STEPHENS

Frei and Stephens have published a paper on the response in the mouse ear to the application of chemicals which are known to be promoters of carcinogenesis. Battelle undertook a preliminary examination of the

effect of painting the ears of mice with smoke condensate. Again, however, the results obtained were not encouraging and this work was also terminated.

GOBLET CELL TEST

In the early part of the year discussions were held with Professor Lynne Reid and Dr. Mawdesley-Thomas on the proliferation of goblet cells which occurs when a rat is exposed to an irritant. At that time it seemed feasible to develop a Goblet Cell Test for the examination of cigarette smoke.

Subsequent work by the Huntingdon Research Centre indicated that the initial promise of the work was not being maintained. This, coupled with the failure of the "Harrogate Smoker" to provide a satisfactory system for exposing rats to smoke, caused further work at Battelle on the development of a test procedure to be suspended. It is possible, however, that the approach might be re-opened if further work by the Huntingdon Research Centre indicates that it is worthwhile.

OUTLINE 1969/70

GENERAL

The current contract with the Battelle Institut expires in March 1970, and to continue the work it will be necessary to negotiate an extension.

For the next few years, the long-term mouse test is likely to remain a major test by which modified cigarettes are judged. It is possible that, in time, this test might be replaced by Inhalation Tests in which animals are exposed to cigarette smoke. However, inhalation tests are not yet developed. For example, a degree of publicity, particularly in the German press, has been given to the inhalation work of Dontenwill at Lokstedt, in which he has exposed hamsters to cigarette smoke. At present, however, he has only produced "tumour-like" changes and not malignant tumours in the hamsters, and it will be some considerable time before a test is developed which could be used to compare the effects of the smoke from different cigarettes.

It seems essential, therefore, that the long-term skin-painting test should be available to B-A.T. As specialised facilities and personnel are required for this type of work, it would be difficult to switch from Battelle in the near future and undertake the work elsewhere.

It would seem worthwhile to allocate approximately 20% of the costs involved in a new contract to allow Battelle to continue to examine samples of cigarettes on a routine basis by the Hyperplasia test, and also to undertake feasibility studies of other tests of interest to B-A.T.

LONG-TERM TESTS

A main aim of undertaking the long-term tests is to provide information to assist in the design of a modified cigarette which has a low mouse-skin response.

From a review of the factors involved in the design of a modified cigarette, it appeared that the work in the immediate future should be concentrated on the examination of sheet materials.

A new experiment (Experiment B8) will be starting in October and involves an examination of PCL from Louisville (X-PCL-5), and a Celanese smoking material (I-308). Cigarettes containing 100% of the sheet materials will be examined, together with cigarettes containing a blend of sheet material and tobaccos.

Also included in Experiment B8 is an examination of the effect of a silica gel filter. A forthcoming publication from Harrogate will show that when a solution of a purified fraction of smoke condensate is passed through a bed of silica gel, then a loss in mouse-skin activity of the solution occurs. Consequently, the silica gel filter is being examined to determine whether a reduction in activity occurs when smoke is passed through a bed of silica gel.

The experiment, therefore, involves six samples:

(i) Control tobacco

(ii) Blend of 40% control tobacco : 60% X-PCL-5

(iii) 100% X-PCL-5

(iv) Blend of 40% control tobacco : 60% I-308

(v) 100% I-308

(vi) Control cigarette with silica gel filter.

An inference which can be drawn from a comparison of the long-term results from Battelle, Harrogate, and Lokstedt, is that the activity of the condensate from a Gerlach sheet is less than that of the condensate from PCL. It is proposed to confirm this inference in a test which will start in March, 1970. At the same time, a PCL sheet incorporating the "Gerlach additives" will be also examined to determine whether these additives lead to an additional reduction in the activity of the condensate.

A second feature of this March experiment is an examination of the effects of an ethanol extraction of tobacco. Lokstedt have shown that extraction with ethanol of a German blend leads to a 35% reduction in the activity of the condensate and it is of interest to determine whether a similar reduction in activity occurs when a flue-cured blend is extracted. An important extention of this work is planned. Extraction with ethanol removes a major portion of the nicotine from the tobacco. It is intended, therefore, to concentrate the extract and add the concentrate back to the tobacco. It is possible that the simple process of "separating" and "recombining" tobacco constituents leads to a reduction in the activity of the condensate.

Finally, it is intended to determine whether the beneficial effect of "extraction" and of "Gerlach process" can be combined, i.e. make a Gerlach sheet from extracted tobacco. In this way it might be possible to obtain a condensate with very low activity.

The samples involved, therefore, in the March experiment are:

(i) Control tobacco

(ii) Gerlach sheet from control tobacco

(iii) PCL sheet from control tobacco, but incorporating the Gerlach additives

(iv) Control tobacco extracted with ethanol

(v) Extracted tobacco with the addition of the concentrated ethanol extract

(vi) Gerlach sheet from extracted tobacco

The Animal House at Battelle has limited capacity and a major factor which determines the starting date of a new experiment is the length of time taken for sufficient mice to die and thereby create space to house a new intake of animals. At the present time, it appears feasible that a third experiment can be started in the later part of 1970.

Experiments completed elsewhere have shown that the addition of sodium nitrate to tobacco leads to a marked reduction in activity, i.e. the incorporation of chemical additives provide the possibility of a "break-through" in the search for a low activity condensate.

The third experiment, therefore, will probably involve the examination of a series of chemical additives. It may be desirable in this experiment to examine the mode of addition of the chemical, e.g. the effect of adding the chemical to the surface of cut tobacco may be different from that produced when the chemical is incorporated into a "PCL mix."

Possible alternatives for this third experiment are a further examination of stem (e.g. WTS on different types of stem) or an examination of an annular cigarette, i.e. a cigarette in which PCL or other material is localised in the axial portion of the cigarette.

HYPERPLASIA TEST

The tests in hand include the examination of a series of filters containing water and samples of cigarettes from Sydney and Louisville. It is anticipated that in the coming year, the heavy demand for samples to be examined will continue.

REPORTS

Title	Report No.
The Tumorigenic Index.	B-11
Hyperplasia Test: Part IV - Progress Report, October 1968.	B-12
Skin-Thickening Test: A Report to T.R.C.	B-13
Further results of work aimed at the development of a Goblet Cell Test	B-14
Mast Cell counts in the skin of mice treated with tobacco smoke condensate.	B-15
Ciliastatsis tests: Part IV - Progress Report, January 1969.	B-16
Ciliastasis tests: Part V - Progress Report, September 1969.	B-17