

5th May, 1960.

Symposium on
"Chemical and Biological Problems related to Smoking"
2nd/3rd May, Stockholm.

The Symposium was arranged by the Advisory Committee of the Swedish Tobacco Monopoly in order to bring together Swedish chemists and physicians interested in the problem and to stimulate new approaches. The programme consisted of lectures by foreign scientists, with open discussions following each paper and the sessions each day were concluded by a number of short communications by Swedish workers, outlining their present researches, most of which were supported by the Swedish Tobacco Monopoly.

Besides the guest lecturers and Swedish workers, there were a number of foreign observers. These included Dr. Barkemeyer (B-A.T., Hamburg) and Dr. Weber, representing the German Research Committee, Dr. Jorgen Lam (Aarhus, Denmark), Dr. Anguere (French Regie), Dr. Day and myself.

The Symposium was opened by Mr. Olof Söderström, the President of the Swedish Tobacco Monopoly, who welcomed the visitors and explained the background to the Swedish work on Smoking and Health. At the request of the Riksdag, the Swedish Medical Research Council was asked in the summer of 1956 to review how the research should be organised to enquire

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into the biological effects of smoke and the chemical analysis of smoke for possible harmful compounds. (The report, made in 1958, was circulated to members of T.M.S.C.) As a result, a Committee was established under the Chairmanship of Prof. Theorell. All projects recommended by the Council have been backed by the Monopoly. A system of joint payment by the Monopoly, the Swedish M.R.C. and the Swedish Cancer Society has been evolved. There is, apparently, a second body, the Scientific Advisory Committee of the Monopoly, on which serve all the members of the M.R.C. Committee, other than Prof. Theorell, and this serves to coordinate the research projects. It is intended to hold other Symposia at later dates, with a purely scientific aim of bringing together interested workers.

The first paper, by Dr. Carruthers (Exeter), was a broad outline of Chemical Carcinogens, tracing the development from Perceval Potts onward and mentioning all types of carcinogens, whether present in tobacco smoke or not. There was no fresh work in this review and the plan of researches at Exeter was not mentioned.

Prof. Druckrey (Freiburg) followed with a paper in which he firstly viewed Carcinogenesis from the point of view of pharmacology. His development of this aspect followed the lines of his paper to the C.I.B.A. Symposium on Carcinogenesis. Thus he referred to the "summation effect" in which response is governed by the sum of the doses. He showed that for 4-dimethylamino stilbene there was a linear relation between daily dose and carcinogenic sum dose and concluded that the life span of the test animal was the only apparent limiting factor governing dose threshold. He then went on to consider smoke condensate and described his laboratory

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work, starting eight or nine years ago. He has used subcutaneous injection of condensate into rats, which were chosen because he now possesses a number of pure strains of known genetic constitution, and this would moreover offer a species different from the mice used by Wynder. This mode of administration was selected because it permits accurate dosage and the possible recognition of systemic effects, unlikely to be noticeable in the purely local effect given by painting techniques.

Denicotinised condensates, collected by cold trap or by electrostatic precipitation, were supplied by Reemtsma and the benzpyrene content was measured by Dr. Grimme in Prof. Tschetsche's laboratory. The solvent, 3-caprylic alcohol, was shown to be free from fluorescent material.

A cumulative dose of 3.2 g. of condensate was applied and treatment then discontinued. Since the carcinogenic action is irreversible, all reversible side effects due to condensate disappear following the cessation of treatment. The animals are then observed over the remainder of their life and histological examination is made of sections taken at post mortem.

Two sorts of condensate were examined

A - normal condensate

B - Condensate from special cigarettes, where efforts were made to ensure as complete combustion as possible i.e. by varying cuts per inch, paper porosity etc.

Malignant tumours were observed in all groups.

<u>Condensate</u>	<u>No. of animals</u>	<u>No. of animals with tumours</u>
A	97	21
B	47	13
None (control)	39	1

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The experiment was repeated on the filial generation and while Condensate A gave an identical result, Condensate B gave only 1 tumour.

Prof. Druckrey concluded that tobacco smoke was very weakly carcinogenic and exerted a purely local action. The total dose corresponded to the condensate from 150 cigarettes per rat, the yield of tumours was small and the induction period prolonged (about 100 weeks for rats, approximately equivalent to 50-60 years in humans). Druckrey believes that for small exposures in humans, the induction period will be greater than the 20-30 years suggested by Doll and others.

A second experiment with the same strains of rats was made using

1. Benzpyrene - 1 μ g/week over the whole life-time
2. Benzpyrene - 11 μ g; anthracene - 10 μ g and pyrene - 10 μ g.
3. 3-Caprylic alcohol

This yielded the following results

<u>Experiment</u>	<u>No. of animals treated</u>	<u>No. of animals with tumours</u>
1	41	2 sarcomata
2	45	4 sarcomata
3	24	2 sarcomata

Druckrey therefore concludes that benzpyrene is not the sole causative agent, since 3.2 g. condensate contains only 3.2 μ g. benzpyrene and yet yields tumours, while 1 μ g. benzpyrene per week for 108 weeks yielded no tumour. He is also following up the idea that tobacco itself is carcinogenic and cited cancer of the mouth in tobacco and betel nut chewers in India. He has begun testing extracts of tobacco made using

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70% alcohol, from which nicotine has not been removed. The dosage is 57 mg./week, using the same subcutaneous route of administration with glycerol as the vehicle. Although the experiments are incomplete, the tumour yield already seems to be greater than with condensate. Druckrey believes that emphasis should be placed primarily on investigation of tobacco leaf, especially the arsenic content, although his reasons were not clear and certainly unconvincing. Nevertheless, his paper was the most stimulating and fresh of the symposium.

Prof. Ingram gave the third paper on electron resonance studies of free radicals produced in tobacco pyrolysis. The first part was a reiteration of the work he carried out jointly with Lyons and already published. He went on to mention a new theory of carcinogenesis, based upon the believed coincidence of upper energy levels between proteins and carcinogenic polycyclic hydrocarbons. He suggested that photoactivation of an electron in a protein to an upper energy level would lead to its transfer to an upper energy level of a hydrocarbon, from which it would drop to a lower level in the hydrocarbon. This lower level would not have an equivalent in the protein, which would therefore now have an unfilled level. His hypothesis was based upon the belief that electrons in the protein would be now delocalised, resulting in a carcinogenetic state.

The theory was attacked by Prof. Theorell who was interested to know how photoexcitation would occur without any noticeable absorption of light of the required energy. Ingram attempted to answer this by saying that the process need not proceed on a scale sufficiently large to be measurable.

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The rest of the session on Monday afternoon was devoted to Swedish contributions. Dr. Westermark suggested that gaseous ions, arising from flames e.g. cooking stoves, electric arc and welding processes, space heaters, car exhausts etc., could lead to a free radical chain reaction, which, occurring in the epithelium of the lung, would be akin to radiation phenomena, (radiomimetic reactions). The theory is based on the assumption that the chain reaction would be carried on through the cell wall to the nucleus. He then described some preliminary experiments in which he measured the magnitude of charges occurring in cigarette smoke. He calculated that, if the charges are radiomimetic, then the dose in cigarette smoke would be large enough to be carcinogenic.

Dr. Dalhamn repeated the paper he gave at Oxford describing his new method of measuring ciliary activity in the rabbit by means of a high speed camera enclosed in a tracheotomy.

Dr. Hökfeld, an endocrinologist, described measurements of the urinary output of aldosterone and hydrocortisone/cortisone made on non-smokers before and after the consumption of a cigarette. The aldosterone level goes down while the cortisone output goes up. He suggested that the nicotine affected the central nervous system, upsetting the control of adrenalin output which in turn controlled the output of ACTH from the adrenal cortex and thereby affected the cortisone level. Alternatively, the effect may be mediated via the hypothalamus. He stated that stress situations led to an increase in aldosterone production and thus nicotine acted to counteract stress. The implications in this work were not made clear by Hökfeld.

Dr. Friberg described the study in progress in collecting data

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on mono and hetero-zygotic twins in Sweden. Data on three quarters of the 95,000 Swedish twins have been entered on punch cards, and interviews have started. Genotype diagnosis is based on the interview in most cases, with an estimated 95% probability of correct diagnosis.

Tuesday 3rd May.

The first session was given by Dr. Wynder who reiterated many of the points he has made on earlier occasions. He stressed that he bases his belief that cigarette smoking is the cause of lung cancer on statistical and epidemiological data, and that the laboratory results are not proof in themselves. He scorned the Fisher theory of genotype difference by referring to his studies on Seventh Day Adventists, and mentioned the recent "cranberry scare" in the U.S. where preventive action was taken based upon one animal experiment and no evidence from humans that aminotriazole was carcinogenic.

He described his animal experiments contrasting the area of the human trachea and bronchi with the area of a mouse skin. Although this lung area is only six times as large as the mouse skin area, it receives about 350 g. condensate per year in contrast to the 10 g. per year administered to mice. He stated that his experiments showed cigarette smoke was weakly carcinogenic to mice.

After referring to the almost complete retention of fluorescent material in the lung on inhaling cigarette smoke, he drew attention to the differing butt lengths in the U.S. and U.K, and congratulated the Swedish Monopoly on the ring printed around their King Size cigarettes, which, he said, was a step in the right direction. Pointing out that there was no evidence

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yet for selective filtration, he decried attempts to rely solely on filters, even of high efficiency, as the solution to the problem.

He then turned to the chemical studies carried out for him by Hoffmann, and called attention to the possible importance of the phenolic fraction as a co-carcinogen or promoter. Without giving any evidence, he suggested that smoke from Virginia type cigarettes may be richer in phenols, perhaps because of a greater content of polyphenols in flue-cured as opposed to air-cured tobaccos. He intends to give this prominence in his research programme. He detailed the chemo-biological studies on condensate, showing how the activity in a rapid test (sebaceous gland suppression) followed benzpyrene, although he admitted that all the known polycyclics in smoke accounted for only about 3% of the biological activity of whole smoke. This was further "evidence" of the existence of a promoter.

He now reports tumours have been obtained with pyrolysates made at 720° and 640°C., as well as at higher temperatures and therefore tended to gloss over the possible effect of combustion temperature upon the formation of biologically effective condensates.

In a new investigation of benzpyrene content of smoke he reports that sidestream smoke contains very large amounts, greater than the main stream. Moreover the smoke from the first half of a cigarette contains 2 ppm benzpyrene whereas that from the second half now only contains 1 ppm. This result has been confirmed in a second experiment. His explanation of this interesting and unexpected finding was extremely muddled, unclear and unconvincing. Wynder appears to be somewhat puzzled himself and did not allude to the opposite result reported by Lindsey.

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He has reopened work on catalysts. Originally he found only copper nitrate led to a reduction in benzpyrene, alumina giving rise to a large increase. Biological activity was only marginally reduced by copper nitrate, magnesium oxide or aluminium silicate. These results were found using 3 puffs per minute. Now, using 1 puff per minute, he finds the biological activity of his control condensate unchanged, but that of condensate from cigarettes with added calcium carbonate and copper nitrate is greatly reduced.

In an endeavour to study in greater detail the dependence of activity upon the type of condensate, he has smoked "spinach cigarettes", which give much greater amounts of benzpyrene, probably owing to the less complete combustion. However tobacco smoke condensate is more active biologically than is spinach smoke condensate. Two differences are the greater basic fraction from tobacco smoke and also the believed greater amount of phenol fraction.

Wynder ended with his usual catalogue of the steps which may be taken to reduce exposure, and mentioned the possibility of selective filtration for the phenol fraction.

It was apparent that while the lecture was "the mixture as before", he has changed his tack somewhat to accommodate the veering wind of emphasis. He is clearly impressed by the new concentration of effort on the phenolic fraction, and without any real weight of observation, is building lofty towers of conjecture upon this foundation.

The second session was given by Dr. Cecilie Leuchtenberger, who described her work on the histopathology of the trachea and bronchi of mice

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exposed to a daily dose of tobacco smoke. Mice were sacrificed after variable exposures and after varying intervals after the cessation of exposure. No changes were noted in the trachea but observations on the bronchi led to sub-division into three categories

- (a) Approximately 50% of the mice showed no reaction.
- (b) About 25% showed a mild bronchitis.
- (c) About 25% showed a very severe bronchitis.

With category (c), Leuchtenberger claimed a marked change in cellular type with proliferation of the epithelium and said that Auerbach agreed that this dysplastic change was identical with carcinoma in situ.

Although the experiment is incomplete, no evidence of an invasive carcinoma has been found, although mice have received the smoke from 1,600 cigarettes. On cessation of smoking, marked desquamation of the epithelium occurs and the state of the bronchi gradually returns to normal.

Irrespective of the dosage (exposure) the same percentages of mice were found to show the three cellular reactions.

This work has been supplemented by analysis for DNA in the cells by microspectrophotometry.

The last session, on Tuesday afternoon, was again devoted to work by Swedish workers. Dr. Welling, of Glasgow working in Prof. von Euler's department, described some approaches towards synthetic analogues of nicotine in which the pyrrolidine ring was opened at the five possible positions. He was followed by von Euler who dealt with the pharmacology of these analogs. His researches to date led him to believe that the biologically active part of the molecule was the N-Methyl pyrrolidine ring, although

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piperidine, isolable from cow and human urine has a nicotine like action on the blood pressure but of only 1/15th the activity. Similarly anabasine is only 1/10th as active as nicotine. None of the synthetic analogues were found to possess more than 1/10th the activity of nicotine and most were inactive.

The tests used were

- (a) Blood pressure of cat.
- (b) Action on rabbit intestinal smooth muscle.
- (c) Action on guinea pig intestinal smooth muscle.

Dr. Skoglund dealt with the effect of nicotine on the central nervous system studied using decapitated cats kept alive by artificial respiration. The drug was administered by intravenous injection or by a special route directly into the spinal cord. The maximum effect was found on monosynaptic reflexes, nicotine appearing to act by depolarising muscle membrane potential. It also affects the output of adrenaline and nor-adrenalin. Dr. Skoglund's thesis was extremely difficult to follow.

Lastly Dr. Claesson (Uppsala) described investigations he has started in the combustion process of tobacco. He is studying shortlived intermediates and, in order to simplify his problem, he is smoking cigarettes in constant (non-intermittent) airflow. Short-lived radicals in the side-stream are measured mass spectrometrically by presentation on a cathode ray oscilloscope which is photographed. Radicals such as methyl etc. in the main stream are measured as methane, ethane etc. by gas chromatography, using a catharometer detector. His chief problem was in ensuring reproducible combustion and this now appears to have been overcome.

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General

On balance, the symposium was rather disappointing as very little new work was reported by the guest lecturers. Much has already been published. The discussions following the papers were not very informative. Perhaps the presence of Dr. Mynder, in particular, served as a "damper" on the proceedings and it was very noticeable that participants tended to seek answers to their questions by personal contacts outside the meetings.

The Swedish Monopoly arranged press conferences before and after the Symposium which were reported in the Swedish press, with photographs of the guest lecturers. Copies of the relevant news-papers were obtained.

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