

September 19, 1974

DISCUSSION OF COLLABORATIVE BIOLOGICAL/BIOCHEMICAL RESEARCH

A meeting took place in this laboratory on September 12, 1974 to discuss possible collaborative research efforts in biology/biochemistry between our Research Dept., McGill University and Dr. Witschi of the Université de Montréal.

<u>PRESENT:</u>	Dr. James C. Hogg]	McGill University
	Dr. S. Inoue]	
	Dr. H. Peter Witschi	Université de Montréal
	Ms. J. Johnson .	
	Dr. M. H. Bilimoria	
	Mr. R. S. Wade	
	Dr. T. A. Smith	

Prior to this meeting, JCH and HPW had never met. RSW, in a brief review, said that B.A.T. were striving to develop good techniques for animal inhalation studies, including the measurement of the extent of penetration of smoke into the lungs. In order to shorten the time needed to assess lung damage, it is proposed to investigate biochemical properties as possible early indicators of damage. Such techniques, if satisfactory, would be extremely useful in facilitating the evaluation of product changes.

Most of the ITPL biochemical work was already known to the visitors, but the unique opportunity is now welcomed to study the relevance of some of the ITPL methods and to extend the search for biochemical indicators of smoke's effects, by the exciting promise of being able to work with animals for the first time, utilizing ideas and suggestions from JCH and HPW.

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The objective of the meeting was to discuss different approaches to the problem and the ways in which such cooperative effort should be planned. It is hoped that the external work will be funded by the CTMC. An outline plan and a rough cost estimate are required by early October for consideration in the new CTMC budget.

JCH has adequate laboratory space for the work, but has little biochemical experience. It is planned that the animal exposure and morphology will be done at McGill, the training in biochemical techniques at U. de M., and the actual experiments after exposures will be done at McGill and at ITPL.

What would be the preferred modus operandum? Dr. Binns would prefer to concentrate on systems which would have relevance to humans. However, HPW thinks this is too idealistic and believes that the best first approach is to use the techniques of classical toxicology to find any indicator of lung damage.

Smoke exposure facilities are a major problem. JCH has a "home-built" machine for exposing guinea-pigs to smoke, but the work really needs equipment suitable for different species of small animals (mouse/rat) which the McGill equipment cannot accommodate. JCH prefers guinea-pigs, because he is interested in lung mechanics, which would be too difficult with smaller animals. HPW's experience to date is mainly with mice and rats. It was agreed that initial work can start with the JCH machine and guinea-pigs, but efforts should be made to obtain a versatile smoker as soon as possible (Oak Ridge or an equivalent one developed at Southampton?). Concerning his home-built smoker, JCH said that he has plenty of evidence that smoke gets into the lung, at least as far as the bronchi and trachea where lesions occur. Besides, HPW and MHB said that it would be better to start with acute studies to find assays of just how much damage smoke does cause and then Southampton could use these to see if any of them could be observable in their long term chronic inhalation studies; thus it was decided that JCH's smoking machine would be quite adequate to begin with since it might take a long time to obtain the Oak Ridge or other machine.

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In discussing the best studies to start the cooperative work, HPW felt that we should not begin with completely new techniques. However, overall HPW felt that one should look for effects in both (a) the lung parenchyma, and (b) the trachea/bronchial tree. He said that two stages were important: 1) The immediate effect on the biochemistry of the lung cells. 2) The effect after 24-48 hours (a lot of tissue repair has normally occurred by then). HPW preferred to work with whole lung homogenates. However, JCH cautioned against a general overview of cells, and was wary of simple inflammation. He prefers to look at separated, cleaned-up cells. There are published techniques on such separation and an expert on these is believed to be coming to Montreal soon to give a paper on them. Both HPW and JCH believe that tracheal cells should not be too hard to isolate.

RESEARCH PROPOSALS

Several areas of research work were discussed, and they are reported below, not arranged in any particular sequence. Later in the day, MHB, JJ and HPW reconvened to work out requirements for numbers of animals and exposures. These have been submitted to JCH for consideration prior to a further meeting of the same group on September 23rd.

1. Transformation of Carcinogens by Aryl Hydrocarbon Hydroxylase (AHH)

MHB asked about the induction of aryl hydrocarbon hydroxylase in animals' lungs by smoke exposure, as HPW is experienced in this work. HPW was not keen on further work on AHH, since he had found that the AHH level in the lungs depends more on the animal's diet than on the smoke dose. Also the AHH approach was receiving so much attention by other workers.

MHB then described the work done by Rosenkrantz and by Ames on the detection of the activity of carcinogens using mutants of E. coli and salmonella respectively, after activation by

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certain enzymes. He suggested that fractions containing AHH, isolated from "smoked" lungs, might enhance the metabolism of carcinogens to "supercarcinogens" (i.e. the other edge of the sword!) in the bacterial mutagenesis tests.

HPW was concerned that such a test system was still further removed from the human situation, but it was pointed out that in fact the salmonella-type test would simply be a useful biochemical method (similar to a chemical end-point) of identifying effects in animal smoke exposure.

JCH was enthusiastic about the use of the salmonella technique, and it was finally agreed that such a study would be worthwhile. Initially MHB would have to learn the AHH isolation technique from HPW. They would then have to find whether AHH could be induced in lungs of guinea pigs using a chemical (cholanthrene). Assuming success in this, animal smoke exposures would be run on JCH's smoker, to find the time after which peak AHH activity was reached. A dose-response study would then be run, sampling always at this peak time. This would then be repeated, and samples isolated not only for AHH activity but also for the mutagenesis study, whereafter a comparison would be possible between smoked and unsmoked control animals, to see the effect of smoke on the lung cell enzymes.

2. Development of Protective Enzyme Systems

It was decided that homogenates of whole lungs would be prepared for measurements of enzymes. The enzymes suggested were:

- superoxide dismutase
- pentose shunt enzymes
- thymidine kinase
- cytochrome oxidase

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3. Biosynthetic Processes in Tracheal Mucosa

Tracheal epithelium cells are of greatest interest, and both the entire trachea and tracheal mucosa should be studied. The guinea pig is not the best animal for this work, but would have to suffice in a relatively specific approach until a versatile smoking machine is obtained.

Suggested studies include:

Incorporation of precursor into glycoproteins

Incorporation of precursor into RNA

Activity of thymidine kinase

Reducing activity (NADPH/NADP ratios)

4. Signs of Lipid Peroxidation in Membranes of Specific Cells

Fluorometric techniques are available for studying lipid peroxidation, although they are tricky to perform. Drugs can be used to proliferate Type II cells, i.e. to "beef" up cells before examination. JCH has definite reservations about lipid peroxidation.

There was some argument about the use of high dose acute toxicology methods, with HPW pro and JCH con. HPW prefers the idea, as a means of short-cutting alot of work. JCH was concerned about exposing guinea pigs too rapidly, since they easily get pneumonia. However, measurement of pneumocytes could well be useful. We should not abandon non-specific methods.

CONCLUSION

Having discussed possible areas of work and listed requirements for them in terms of numbers of animals and exposures, the objective of the meeting on September 23 is to set priorities for the work and prepare a draft programme with rough costs, for submission to CTMC.

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