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PULMONARY ARYL HYDROCARBON HYDROXYLASE: TOBACCO  
SMOKE-EXPOSED GUINEA PIGS

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A CONSIDERABLE AMOUNT OF WORK HAS BEEN CARRIED OUT AT THE PATHOLOGY INSTITUTE TO TRY AND UNDERSTAND THE MECHANISM BY WHICH CIGARETTE SMOKE CAUSES TOXIC DAMAGE TO THE RESPIRATORY TRACT OF GUINEA PIGS. INITIAL PHYSIOLOGIC STUDIES INVOLVING ACUTE EFFECTS OF CIGARETTE SMOKE ON LUNG FUNCTION IN GUINEA PIGS SHOWED THE ANIMALS DID IN FACT INHALE SMOKE INTO THE TRACHEOBRONCHIAL TREE WHEN THEY WERE EXPOSED IN THE SMOKING MACHINE. NEXT, ELECTRON MICROSCOPIC STUDIES REVEALED THAT SMOKE DID INTERFERE WITH THE NORMAL PROTECTIVE FUNCTION OF THE

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RESPIRATORY EPITHELIUM, POSSIBLY BY LOOSENING OR OPENING OF THE EPITHELIAL TIGHT JUNCTIONS, AND THAT THIS CHANGE OCCURRED BEFORE THERE WAS ANY METAPLASTIC OR NEOPLASTIC CHANGE IN THE EPITHELIUM. IN THESE STUDIES IT WAS SHOWN THAT ON EXPOSURE TO 5 CIGARETTES A DAY FOR 10 DAYS, THE SMOKE WAS ABLE TO PENETRATE INTO THE BRONCHIOLI AND ALVEOLI, WHILE ON EXPOSURE TO 5 CIGARETTES A DAY FOR 50 DAYS, IT WAS ALSO ABLE TO PENETRATE INTO THE TRACHEAE.

ON ACCOUNT OF THE AVAILABILITY OF THIS USEFUL PHYSIOLOGICAL AND MORPHOLOGICAL INFORMATION ON THE SMOKE-EXPOSED GUINEA PIGS WE DECIDED TO SELECT THIS ANIMAL MODEL TO STUDY SOME BIOCHEMICAL EFFECTS OF CIGARETTE SMOKE. ONE WELL-KNOWN BIOCHEMICAL EFFECT OF SMOKE INHALATION IS THE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE. THIS MIXED FUNCTION OXIDASE APPEARS TO PLAY A ROLE IN CARCINOGENESIS, POSSIBLY PLAYING A DUAL ROLE IN DRUG METABOLISM, AND THEREFORE, WAS THE PARAMETER OF CHOICE FOR INITIATING OUR BIOCHEMICAL STUDIES. INTEREST IN THIS ENZYME COMPLEX HAS BEEN HEIGHTENED RECENTLY BY ITS USE AS AN IN VITRO ACTIVATION SYSTEM IN THE SALMONELLA TYPHIMURIUM TEST DEVELOPED BY AMES TO DEMONSTRATE MUTAGENIC ACTIVITY OF CARCINOGENS.

RANDOM BRED ADULT GUINEA PIGS OF EITHER SEX AND WEIGHING BETWEEN 300-400 GM. WERE PUT IN A CHAMBER

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CONNECTED TO A SMOKING DEVICE DESIGNED IN THE INSTITUTE WORKSHOP. FOR INHALATION STUDIES EMPLOYING SPRAGUE-DAWLEY RATS, THE CHAMBER WAS APPROPRIATELY MODIFIED. THE ANIMALS WERE ALWAYS EXPOSED TO SMOKE FROM 5 CIGARETTES (CANADIAN FLUE-CURED BLEND), WITH A BREAK OF 5 MINUTES BETWEEN EACH CIGARETTE. THE ANIMALS WERE SACRIFICED AND THE ORGANS EXCISED, WEIGHED AND HOMOGENISED IN 3 VOLUMES OF 0.15M KCL - 0.05 TRIS BUFFER PH 7.5, USING A POLYTRON (BRINKMANN INSTRUMENTS LTD.). THE AHH ACTIVITY WAS MEASURED IN TOTAL LUNG OR LIVER HOMOGENATES.

A SURVEY OF THE LITERATURE REVEALED THAT TOBACCO OR TOBACCO SMOKE CONDENSATE INCREASES AHH ACTIVITY IN THE LUNGS OF MICE, RATS AND HAMSTERS, HENCE, WHEN WE EXPOSED GUINEA PIGS TO CIGARETTE SMOKE, WE EXPECTED TO OBTAIN INCREASED LEVELS OF THIS ENZYME IN THE LUNGS. HOWEVER, TO OUR SURPRISE, THIS WAS NOT SO, AND WE FOUND THAT CIGARETTE SMOKE FAILED TO INDUCE AHH ACTIVITY IN GUINEA PIG LUNG. THIS IS SHOWN CLEARLY IN FIGURE 1 IN WHICH ARE RECORDED THE RESULTS OF A DETAILED TIME COURSE STUDY. THIS STUDY WAS CARRIED OUT TO ENSURE THAT WE MEASURED AHH ACTIVITY AT THE TIME PERIOD OF PEAK INDUCTION AFTER EXPOSURE TO SMOKE. FROM THE FIGURE IT WILL BE SEEN THAT INSTEAD OF OBTAINING INCREASED ACTIVITIES, EXPOSURE TO CIGARETTE SMOKE RESULTED

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IN A SIGNIFICANT LOWERING OF ACTIVITY AT TIME PERIODS UP TO 12 HOURS. IT MAY BE MENTIONED THAT AT 3 HOURS THERE NEVER WAS, IN REPEATED EXPERIMENTS, ANY DIFFERENCE BETWEEN SMOKE-EXPOSED AND CONTROL ANIMALS. FOR THIS WE HAVE NO READY EXPLANATION. AFTER 24 HOURS AND UPTO 3 DAYS, THE LEVELS OF PULMONARY AHH ARE THE SAME IN BOTH CONTROL AND SMOKE EXPOSED ANIMALS.

NEXT SLIDE PLEASE. FIGURE 2

FIGURE 2 RECORDS TOTAL ACTIVITIES OF PULMONARY AHH IN CONTROL AND SMOKE EXPOSED GUINEA PIGS. ONCE AGAIN IT WILL BE SEEN THAT CIGARETTE SMOKE DOES NOT INDUCE AHH IN THE GUINEA PIG LUNG. TOTAL ACTIVITIES IN TERMS OF NANOGRAMS 3-OH-BENZOPYRENE FORMED PER MINUTE PER TOTAL LUNG WERE ALSO CALCULATED, SINCE IT HAS BEEN POINTED OUT THAT ABNORMAL INCREASE IN PULMONARY PROTEINS, SUCH AS THAT PRODUCED IN EDEMA AND CELLULAR INFILTRATION, MIGHT GIVE ARTIFICIALLY LOW SPECIFIC ACTIVITIES OF THE ENZYME. FROM THE FIGURE, IT WILL BE SEEN THAT THE SMOKE EXPOSED ANIMALS ALWAYS SHOW LOWER ACTIVITIES THAN CONTROLS UPTO 12 HOURS AFTER SMOKE EXPOSURE, ALTHOUGH THE DIFFERENCES ARE NOT ALWAYS SIGNIFICANT AT THE 0.05 LEVEL OF SIGNIFICANCE. ONCE AGAIN, AFTER 24 HOURS AND UPTO 3 DAYS, THE LEVELS OF PULMONARY AHH ARE THE SAME IN BOTH CONTROL AND SMOKE EXPOSED ANIMALS.

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NEXT SLIDE PLEASE. TABLE 1

TABLE 1 SHOWS THE SPECIFIC ACTIVITIES OF AHH IN THE LIVERS OF CONTROL AND SMOKE-EXPOSED GUINEA PIGS. THERE IS NO SIGNIFICANT DIFFERENCE IN THE LEVELS OF AHH IN THE LIVERS OF THE TWO GROUPS OF ANIMALS. THIS IS TO BE EXPECTED SINCE NO STUDY THUS FAR HAS SHOWN INDUCTION OF AHH IN THE LIVER AS A RESULT OF SMOKE EXPOSURE. HOWEVER, A RECENT STUDY, REPORTED SINCE THIS WORK WAS CARRIED OUT, SHOWS THAT WHILE SMOKE EXPOSURE DOES NOT INDUCE AHH IN THE LIVER AND INTESTINE OF SPRAGUE-DAWLEY RATS AND SEVERAL MICE STRAINS STUDIED, IT DOES SO IN THE LUNGS OF BOTH RATS AND MICE (INDUCIBLE AND NON-INDUCIBLE STRAINS), AS ALSO IN THE KIDNEY OF SPRAGUE-DAWLEY RATS AND OF "INDUCIBLE" STRAINS OF MICE.

NEXT SLIDE PLEASE. TABLE 2

IN THIS EXPERIMENT WE EXPOSED THE GUINEA PIGS TO THE SMOKE FROM 5 CIGARETTES DAILY FOR 41 DAYS TO FIND OUT IF THIS INCREASED EXPOSURE RESULTED IN THE INDUCTION OF PULMONARY AHH. FROM THE TABLE IT WILL BE SEEN THAT EVEN THIS GREATER EXPOSURE FAILED TO INDUCE THE ENZYME. IN FACT, THERE WAS, ONCE AGAIN, A LOWERING OF AHH IN THE LUNGS OF THE SMOKE-EXPOSED GUINEA PIGS.

NEXT SLIDE PLEASE. TABLE 3

SINCE THE NEGATIVE RESULTS OBTAINED WITH RESPECT

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TO INDUCTION OF AHH IN GUINEA PIG LUNG WERE UNEXPECTED, WE FELT IT IMPORTANT TO VERIFY THE RESULTS BY SOME ADDITIONAL EXPERIMENTS. THE FIRST POINT TO BE CHECKED WAS THE EXPOSURE SYSTEM. TO DO THIS WE EXPOSED SPRAGUE-DAWLEY RATS TO SMOKE FROM 5 CIGARETTES IN THE SAME SMOKING MACHINE, AND THE RESULTS OBTAINED WERE UNEQUIVOCAL" 3 HOURS LATER, AHH ACTIVITY IN LUNG WAS MORE THAN 4 TIMES AS HIGH AS IN THE LUNGS OF CONTROL ANIMALS. THIS EXPERIMENT PROVED THAT OUR CONDITIONS OF SMOKE EXPOSURE WERE ADEQUATE TO REPRODUCE WHAT HAD BEEN FOUND BY EARLIER INVESTIGATORS, THAT CIGARETTE SMOKE DID INDEED INCREASE THE ACTIVITY OF PULMONARY AHH IN RATS.

NEXT SLIDE PLEASE. TABLE 4

THE NEXT STEP WAS TO ASCERTAIN WHETHER KNOWN INDUCERS OF PULMONARY AHH IN RATS WERE ALSO CAPABLE OF INCREASING PULMONARY AHH IN GUINEA PIGS. THE AGENTS CHOSEN WERE 3-METHYLCHOLANTHRENE AND ARACHLOR 1254, BOTH OF WHICH ARE KNOWN TO INCREASE PULMONARY AND HEPATIC AHH ACTIVITIES IN RATS SEVERAL FOLD OVER BASAL LEVELS AFTER A SINGLE INTRAPERITONEAL INJECTION. FROM THE TABLE IT WILL BE SEEN THAT WHILE 3-METHYLCHOLANTHRENE INDUCES PULMONARY AHH IN THE SPRAGUE-DAWLEY RAT, IT FAILS TO DO SO IN THE GUINEA PIG. ARACHLOR, TOO, INDUCES PULMONARY AHH IN THE RAT, BUT UNLIKE 3-METHYLCHOLANTHRENE, WHICH HAD NO EFFECT ON GUINEA PIG PULMONARY AHH, ARACHLOR DEPRESSES PULMONARY AHH IN GUINEA VERY MARKEDLY. THIS

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IS REMINESCENT OF THE MARKED DEPRESSION OF PULMONARY AHH IN GUINEA PIGS SEEN WITH TOBACCO SMOKE.

NEXT SLIDE PLEASE. TABLE 5

IN THIS TABLE ARE SHOWN THE RESULTS OBTAINED WHEN GUINEA PIGS AND RATS ARE INJECTED INTRAPERITONEALLY WITH 3-METHYLCHOLANTHRENE AND ARACHLOR 1254, AND LEVELS OF HEPATIC AHH MEASURED. FROM THE TABLE IT WILL BE SEEN THAT BOTH THESE AGENTS ARE INDUCERS OF HEPATIC AHH IN BOTH SPECIES. THUS, WHILE ARACHLOR AND 3-METHYLCHOLANTHRENE FAILED TO INDUCE PULMONARY AHH IN GUINEA PIG BOTH AGENTS DO INDUCE HEPATIC AHH IN THIS SPECIES, THUS CONFIRMING THE OBSERVATION MADE IN ANOTHER STUDY THAT HEPATIC AHH IS INDUCIBLE IN THE GUINEA PIG.

NEXT SLIDE PLEASE. TABLE 6

THE DEPRESSION IN AHH UPTO 12 HOURS AFTER SMOKE INHALATION BY GUINEA PIGS, IT WAS FELT, MAY BE DUE TO THE EFFECT OF CARBON MONOXIDE AND CYANIDE PRESENT IN THE GASEOUS PHASE OF CIGARETTE SMOKE. SINCE THESE COMPOUNDS ARE KNOWN TO COMPLEX WITH CYTOCHROME P-450, SUCH AN INHIBITION WOULD NOT BE SURPRISING. IN THIS EXPERIMENT THE GUINEA PIGS WERE MADE TO INHALE GASES PASSING THROUGH A GLASS FIBRE FILTER (CAMBRIDGE FILTER) PLACED BETWEEN THE BURNING CIGARETTES AND THE INHALATION CHAMBERS. THE FILTER RETAINS THE PARTICULATES LARGER THAN 0.1 U IN DIAMETER AND ALLOWS THE GASES INCLUDING CO AND A LARGE

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PROPORTION OF THE HCN THROUGH. THE INHALATION OF THE VAPOUR PHASE DOES NOT DEPRESS LEVELS OF PULMONARY AHH, SHOWING THAT THE COMPONENTS RESPONSIBLE FOR THE DEPRESSION IN AHH ACTIVITY AFTER SMOKE INHALATION RESIDE IN THE PARTICULATE MATTER.

### CONCLUSIONS

FROM OUR STUDY WE WOULD LIKE TO DRAW THE FOLLOWING CONCLUSIONS.

1. THE VAPOUR PHASE OF CIGARETTE SMOKE ALONE DOES NOT DEPRESS AHH ACTIVITY IN GUINEA PIG LUNGS. THIS IS AN IMPORTANT OBSERVATION. FROM THIS EXPERIMENT WE MAY INFER THAT THE DEPRESSION OF AHH IS DUE TO SOME AGENT IN THE PARTICULATE MATTER OF CIGARETTE SMOKE. IT IS THOUGHT BY MOST WORKERS IN THE FIELD THAT THE ACUTE AND/OR CHRONIC EFFECTS OF CIGARETTE SMOKE ARE DUE TO AGENTS PRESENT IN THE PARTICULATE PHASE. OUR DATA SUGGEST THAT SUCH DAMAGE MIGHT ALSO INCLUDE DEPRESSION OF AHH.

2. IN ALL STUDIES REPORTED SO FAR WITH OTHER SPECIES, THIS DEPRESSION WAS EITHER NOT SEEN OR QUICKLY SUPERCEDED BY INDUCTION. ONLY IN GUINEA PIGS, WHERE THERE IS APPARENTLY NO INDUCTION POSSIBLE, IS SUPPRESSION A PROMINENT FEATURE OF ACUTE SMOKE TOXICITY. MOST CHRONIC INHALATION STUDIES WITH TOBACCO SMOKE ARE CONDUCTED WITH

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INDUCIBLE ANIMAL SPECIES - RATS, MICE AND HAMSTERS. IT MIGHT, THEREFORE, BE WORTHWHILE TO INCLUDE THE GUINEA PIG IN CHRONIC STUDIES ON TOBACCO SMOKE INHALATION.

3. THERE IS CURRENTLY A GREAT DEAL OF INTEREST IN DETECTING INDIVIDUALS AT RISK TOWARDS THE CARCINOGENIC POTENTIAL OF INHALED TOBACCO SMOKE. ONE WAY TO IDENTIFY SUCH INDIVIDUALS IS TO MEASURE IN VITRO AHH INDUCIBILITY IN LYMPHOCYTES. SINCE WE CANNOT COMPARE INDUCIBILITY IN LYMPHOCYTES WITH INDUCIBILITY IN HUMAN LUNG TISSUE, ANIMAL MODELS ARE NEEDED IN ORDER TO TEST A POSSIBLE CORRELATION. MOUSE STRAINS SHOWING DIFFERENTIAL INDUCIBILITY AND DIFFERENTIAL SUSCEPTIBILITY AGAINST POLYCYCLIC HYDROCARBONS ARE ONE OBVIOUS EXPERIMENTAL MODEL. GUINEA PIGS COULD SERVE AS ANOTHER ONE, A NEGATIVE CONTROL. IF A CORRELATION BETWEEN LYMPHOCYTE AND PULMONARY AHH ACTIVITY EXISTS, THEN GUINEA PIG LYMPHOCYTES AHH ACTIVITY SHOULD NOT BE INDUCIBLE IN VITRO. WE HOPE TO SHOW SOON WHETHER THIS IS INDEED TRUE OR NOT.

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