Final Report
Contract A6-104-30

INDUCTION OF ARYL HYDROCARBON HYDROXYLASE IN CULTURED PERIPHERAL LYMPHOCYTES IN COLLEGE STUDENTS EXPOSED TO AIR POLLUTANTS

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ABSTRACT

Polycyclic aromatic hydrocarbons are known environmental contaminants and are metabolized to active carcinogenic forms by aryl hydrocarbon hydroxylase. In this study it was hypothesized that exposure to heavy air pollutants would result in higher levels of aryl hydrocarbon hydroxylase activity. To test this hypothesis buffy coat cells were isolated from peripheral blood obtained from students residing at two Seventh-day Adventist colleges. One college, Riverside Campus of Loma Linda University, represents a high air pollution exposure site and the other, Pacific Union College, Angwin, CA, represents a low air pollution exposure site. Blood samples were obtained from the same individuals on three different occasions. At each drawing white blood cells were cultured in the presence of mitogens for 48 hours. Cells were then harvested and frozen at -80°C until the enzyme assays were performed. The enzyme assay involved the spectrophotofluorometric detection of phenolic products when samples of cultured cells were incubated in the presence of benzo(a)pyrene. Data are expressed as units per mg cell protein.

Results obtained indicate wide interindividual variation irrespective of residence. Seasonal variations are also indicated. Significantly higher mean units of aryl hydrocarbon hydroxylase activity are associated with those students residing at the Riverside Campus of Loma Linda University. Differences are significant at a p value less than 0.0001. These results suggest that under the conditions employed in this study higher levels of aryl hydrocarbon hydroxylase activity are found in individuals living in geographical regions with high levels of air pollution.

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DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

CONCLUSIONS

Data obtained in this study suggest that metabolism of polycyclic aromatic hydrocarbons differs between human populations exposed to low air pollution levels and those exposed to high air pollution levels. Measurements of aryl hydrocarbon hydroxylase in cultured lymphocytes isolated from student blood samples show large interindividual variation within each population irrespective of the degree of air pollution exposure. When mean values of aryl hydrocarbon hydroxylase activity are compared significant differences exist between the low smog and high smog groups. The greatest difference between these two populations occurred in the 1977 fall assay following the summer season of high smog levels in the south coast basin. This assumes that levels of polycyclic aromatic hydrocarbons correlate directly with smog levels but since the air pollution monitoring stations employed did not register polycyclic aromatic hydrocarbons it is impossible to correlate seasonal values of aryl hydrocarbon hydroxylase activity with levels of polycyclic aromatic hydrocarbons to which the students in this study were exposed.

Although steps were taken to minimize as many variables as possible in this study it is still possible that some as yet undefined laboratory or environmental factor may be responsible for the differences observed and reported here.

RECOMMENDATIONS

As a result of this pilot study it is recommended that a follow-up study be implemented. This study should incorporate the following refinements.

- 1. The air pollution monitoring facilities on both college campuses should be modified to measure levels of polycyclic aromatic hydrocarbons (benzo(a)pyrene) in ambient air.
- 2. Students should be pre-screened to eliminate as many variables as possible and fewer numbers of students should be employed.
- 3. To minimize variability assays should be performed on cultured monocytes that are isolated from student blood samples.
- 4. A more sensitive spectrophotofluorometric instrument should be utilized during assays to determine aryl hydrocarbon hydroxylase activity.
- 5. Assays should be performed on monocytes isolated from student blood samples at four different times. At the beginning of the academic year as students arrive on campus, at the beginning of the winter quarter and spring quarter and finally at the beginning of the fall quarter of the following academic year. This will make it possible to follow each student through the course of one full year.
- 6. Using high pressure liquid chromatographic techniques the metabolic profile of randomly selected cultures should be analyzed to determine whether the metabolic products include active carcinogenic compounds.

INTRODUCTION

Polycyclic aromatic hydrocarbons are recognized environmental contaminants. They are released into the environment with the incomplete combustion of organic material. Polycyclic aromatic hydrocarbons (PAH) are present then in cigarette smoke, burning refuse, automobile exhaust emissions and in emissions from the making of coke or from oil refineries (1). Studies of exposure of humans to polycyclic aromatic hydrocarbons is limited primarily to smokers and to workers in selected occupations. PAH emissions from motor vehicles are substantially reduced by the statutory standards for lowering the content of carbon monoxide and hydrocarbons in exhaust emissions (2). In calculations of human exposure to benzo(a)pyrene (BP), a commonly monitored polycyclic aromatic hydrocarbon, values are highest for coke oven workers and coal tar pitch workers (2). Although BP emissions from motor vehicles represents a relatively small source compared to other sources they may be important in the total exposure to the nonsmoking commuter.

Carcinogenic effects of polycyclic aromatic hydrocarbons have been recognized for years and a voluminous amount of reference material is available which describes in vivo and in vitro studies of chemical carcinogenesis. Benzo(a)pyrene is a representative PAH whose chemical and biological action has been studied extensively. BP is a recognized air pollutant and it has been estimated that 2000 tons of BP are emitted into the atmosphere over the United States each year (1). BP is not the ultimate carcinogen but must be metabolized to form active intermediates which act as the ultimate carcinogen. Evidence suggests that the ultimate carcinogenic agent binds to cellular macromolecules (DNA, RNA, protein) to alter cellular function and induce the abnormal changes associated with cancer. The intermediates of BP metabolism that are suspect include arene oxides and diol epoxides (3).

Polycyclic aromatic hydrocarbons including BP are metabolized by a membrane associated mixed function oxygenase enzyme called aryl hydrocarbon hydroxylase (AHH) (4). The AHH enzyme system may play a key role in the metabolic activation of PAH substances and in the carcinogenic process. Exposure to polycyclic aromatic hydrocarbons induces AHH activity in most mammalian tissues (5) including cultured human lymphocytes (6), monocytes (7), and pulmonary macrophages (8).

Since benzo(a)pyrene is a recognized environmental pollutant and its presence is associated with heavily industrialized urban settings it was proposed that a study of the induction of aryl hydrocarbon hydroxylase in cultured human lymphocytes be undertaken. In this study a comparison is made between two groups. One represents exposure to low ambient air pollutants and the other exposure to high air pollution. An initial basic assumption in the design of this study was that the level of air pollution will also reflect differences in the exposure to polycyclic aromatic hydrocarbons and that this difference in exposure to PAH will be reflected in measured differences in the activity of the AHH enzyme system in cultured peripheral lymphocytes in the two populations. To minimize variables and to select as homogeneous a population as possible it was proposed that blood samples be

taken from freshman college students on two Seventh-day Adventist undergraduate campuses. Pacific Union College, Angwin, California represents the low smog environment and the Riverside Campus of Loma Linda University represents the high smog environment. Health standards adopted by Seventh-day Adventists includes abstinence from the use of tobacco, drugs and alcoholic beverages. Since AHH enzyme activity is affected by a variety of chemical agents including tobacco, drugs and alcohol the selection of subjects from these two college campuses should provide relatively homogeneous groups with the important variable being the air pollution level.

MATERIALS AND METHODS

Peripheral blood was obtained in heparinized vacutaners from individual students on each college campus. In 1976 individual samples were collected on the dates November 7-10 at Pacific Union College and November 14-17 at Loma Linda University, Riverside Campus. Two collections were made in 1977 at each campus, April 10-13 and September 23-29 at Pacific Union College and April 17-20 and September 30 - October 5 at Loma Linda University, Riverside Campus. Samples collected at Pacific Union College were flown by private plane to the Loma Linda Campus of Loma Linda University.

Upon arrival blood samples were centrifuged at 420Xg for 6 minutes and the serum was discarded. The buffy coat layer containing the lymphocytes was removed and placed in a sterile 5 ml disposable centrifuge tube. Contaminating red blood cells were lysed by adding 3 ml ACK lysing buffer (9) with gentle agitation at 4°C for 4 min. Cells were then centrifuged at 180Xg and the supernatant was discarded. The cell pellet was resuspended in 20 ml RPMI 1640 culture medium (Grand Island Biological Co.) containing 15 percent fetal calf serum (Grand Island Biological Co.), 1% phytohemagglutinin (PHA), 1% pokeweed mitogen (PWM), 70 μ g/ml heparin and antibiotics. PHA, PWM and antibiotics were purchased from Grand Island Biological Co. Heparin was obtained from Sigma Chemical Co. Lymphocytes were cultured in 25 cm² tissue culture flasks (Corning) at 37°C for 48 hrs.

Lymphocyte cultures were harvested after 48 hrs by centrifugation at $250 {\rm Mg}$. The supernatant was discarded and the cells were washed twice in chilled Moscona's saline (10). The cells were pelleted by centrifugation at $180 {\rm Mg}$ and frozen as a pellet in 1 ml of TMS buffer at $-80 {\rm ^{\circ}C}$. TMS buffer contains $50 {\rm ^{m}}$ Moles Tris - HCl (pH 7.5), $3 {\rm ^{m}}$ Moles MgCl₂ and 0.2 M sucrose (6).

Assays for aryl hydrocarbon hydroxylase activity were determined by a modified procedure (4,6). Frozen samples were thawed and cells were resuspended in 1.75 ml TMS buffer. A 0.25 ml aliquot was removed for protein determination. Assays on whole cell samples (11) were run in duplicate. Each sample was incubated for 30 min at 37°C in a final volume of 1.1 ml containing 1.0 mg of reduced nicotinamide adenine dinucleotide (Sigma Chemical Co.) and 25 μg of benzo(a)pyrene. The reaction conducted in the dark was stopped by the addition of 4 ml of an acetone, hexane mixture (1:3) and shaking for 3 min. The phases were separated by centrifugation at 180Xg and 1 ml of the

organic phase was extracted with 3 ml of 1 N NaOH. The aqueous and organic phases were separated by centrifugation (180Xg) and fluorescence in the aqueous phase was determined spectrophotofluorometrically (4). Measurements were compared to those of a standard 3-hydroxybenzo(a)pyrene solution. Values were corrected by subtracting the fluorescence of a reaction mixture containing all the component chemicals but no cells. AHH enzyme activity was expressed as units per mg cell protein where a unit is defined as that catalyzing in 30 min the formation of phenolic products with fluorescence equal to 1 p mole of 3-hydroxybenzo(a)pyrene. Cellular protein was determined by a modification of the Wu-Herriott procedure (12).

Nontechnical Description of Culture and Analysis Procedures

The buffy coat layer containing the lymphocytes was carefully removed from each centrifuged blood sample. The cells in this layer were resuspended in culture medium containing 15 percent fetal calf serum, 1% phytohemagglutinin, 1% pokeweed mitogen, heparin and antibiotics. The chemicals phytohemagglutinin and pokeweed mitogens act to stimulate cell division in lymphocytes. The blood cells were kept in an incubator at 37°C for 48 hrs. After 48 hrs the culture medium from each flask was centrifuged and the cells growing in the medium were washed in a buffer solution and frozen at -80°C until the assay for aryl hydrocarbon hydroxylase was performed. Assays for the enzyme aryl hydrocarbon hydroxylase (AHH) were performed on thawed cell samples by incubating the cells in the presence of benzo(a)pyrene for 30 min. The metabolic products were extracted with NaOH and the fluorescence of these phenolic products was then measured and compared to a standard 3-hydroxybenzo(a)pyrene fluorescence curve. AHH activity was expressed as units per milligram of cell protein. Cell protein determinations were made by reacting small aliquots of cell samples with diphenylamine. The intensity of the color reaction was then measured and the results were read from a standard curve.

RESULTS

Table I illustrates aryl hydrocarbon hydroxylase activity in cultured lymphocytes obtained from peripheral blood from students residing at the campus of Pacific Union College, Angwin, California. This private parochial college is geographically located in a low air pollution setting. Mean values of AHH activity expressed as units per mg cell protein are shown for samples assayed in the spring and fall of 1977. Questionnaires obtained from each student at each blood drawing were analyzed. Students who were sick, on medication within 4 weeks of the blood drawing or who were frequent or occasional smokers of either tobacco or marijuana are classified as a separate group in each table. Group A includes all student samples assayed. Group B includes only data from students who were sick, on medication or smoking. Group C is composed of data from those students in Group A that are not included in Group B. In the fall data shown in Table I Group B also includes those students who were in a high air pollution environment during the last 4 weeks of the summer of 1977. Differences between mean $(\bar{\mathbf{x}})$

values of AHH activity of all samples and those of the other two categories in Table I are not significant at a p=0.05 level. This is true for both the spring and fall assays. Differences in mean values between any comparable group assayed in the spring as compared with that in the fall are not significant at the p=0.05 level.

Table I. AHH activity in cultured lymphocytes obtained from peripheral blood from students at Pacific Union College. Blood samples were drawn in April and September, 1977. Group A includes all student samples. Group B includes students who were sick, on medication or smoking. Group C represents those students in Group A that are not included in Group B.

		Spring			Fall	
GROUP	Иа	Σ̄b	S.E.M.b	Na	Σp	S.E.B.b
	184	5.22	0.34	136	5.34	0.45
	91	5.1	0.5	87	5.36	0.5
	93	5.34	0.48	49	5.29	0.59

^aN = number of samples used to compute the mean. Lymphocyte samples from each student were assayed in duplicate and averaged. Actual number of samples assayed is twice N.

Table II shows aryl hydrocarbon hydroxylase activity in cultured lymphocytes obtained from peripheral blood from students at the Loma Linda University, Riverside Campus, Riverside, CA. This college is geographically situated in the south coast basin in a high air pollution setting. Students who were sick, on medication, or smoking during the four weeks prior to the blood drawing are categorized separately in Group B. In the fall data shown in Table II Group B also includes those students who were in a low air pollution environment during the last 4 weeks of the summer of 1977. The statistical significance of differences between groups in the spring assay is equivocal and is not significant at the p = 0.05 level. A significant difference does exist when groups are compared between the spring and fall assays.

^bMean(\bar{x}) and standard error of the mean (S.E.M.) values are expressed as units per mg cell protein. A unit is defined as that catalyzing in 30 min the formation of phenolic products with fluorescence equal to 1 p mole 3-hydroxybenzo(a)pyrene. Culture and assay procedures are described in Materials and Methods.

Table II. AHH activity in cultured lymphocytes obtained from peripheral blood from students at Loma Linda University, Riverside Campus. Blood samples were collected in April, late September and early October, 1977. Group A includes all student samples. Group B includes students who were sick, on medication or smoking. Group C includes those students in Group A that are not included in Group B.

·		Spring			Fall	
GROUP	 . Na	χ̄b	S.E.M.b	Nа	Σp	S.E.M.b
A B C	175 73 102	6.86 6.05 7.45	0.32 0.47 0.43	183 109 74	9.77 9.8 9.86	0.49 0.63 0.77

 $^{^{}a}N$ = number of samples used to compute the mean. Lymphocyte samples from each student were assayed in duplicate and averaged. Actual number of samples assayed is twice N.

In Table III mean values of AHH activity are compared between lymphocyte samples assayed from students representing the low air pollution environment at Pacific Union College and those from the high air pollution environment at Loma Linda University, Riverside Campus, for both the spring and fall blood drawings. Differences between values obtained at each campus for both the spring and fall assays are significant at p values less than 0.0001. The difference is particularly striking when comparisons of the fall assay are made.

bMean (x) and standard error of the mean (S.E.M.) values are expressed as units per mg cell protein. A unit is defined as that catalyzing in 30 min the formation of phenolic products with fluorescence equal to 1 p mole of 3-hydroxybenzo(a)pyrene. Culture and assay procedures are described in Materials and Methods.

Table III. A comparison of AHH activity of all lymphocyte samples assayed from students at Pacific Union College with those from Loma Linda University, Riverside Campus for both the 1977 spring and fall blood drawings.

College	Sp	ring	F	all
Represented	z a	S.E.M.a	χ̄a	S.E.M. ^a
Pacific Union Angwin, CA	5.22	0.34	5.34	0.45
Loma Linda Riverside, CA	6.86	0.32	9.77	0.49

Mean \bar{x} and standard error of the mean (S.E.M.) values are expressed as units per mg cell protein. A unit is defined as that catalyzing in 30 minutes the formation of phenolic products with fluorescence equal to 1 p mole of 3-hydroxybenzo(a)pyrene. Culture and assay procedures are described in Materials and Methods.

AHH activity is not given for samples collected in November of 1976 since technical difficulties invalidates the data obtained.

DISCUSSION

Measurements of aryl hydrocarbon hydroxylase activity is an index of the capacity of cells and tissues to metabolize polycyclic aromatic hydrocarbons. PAH compounds are recognized environmental contaminants and members of this group are known carcinogens. Studies of carcinogen metabolism in humans have been accomplished using cultured lymphocytes isolated from blood (6,7). The significance of PAH metabolism is under investigation in a number of laboratories. Studies indicate that a number of metabolic intermediates have enhanced carcinogenicity over that of the parent compound (13). Since humans are exposed to these environmental contaminants studies of metabolic activation using human cells are necessary. Data suggest that AHII induction in humans is under genetic control (14) and that genetic rather than environmental factors are responsible for the interindividual variability (15).

Individual data of AHII activity as reported in this study (Appendix) shows tremendous variability. This has been reported by others (15,16). Seasonal variations also occur with higher AHH activity occurring in the summer and fall (17). Seasonal variation is seen in comparing the AHH activity of samples assayed in the spring with those assayed in the fall for students at Loma Linda University, Riverside Campus (Table II) but not

for students at Pacific Union College (Table I). Although benzo(a)pyrene was not monitored by the air monitoring stations located on these two college campuses it is assumed that levels of PAH compounds in the atmosphere correlate with smog levels. Particularly in the south coast basin the summer season is marked by high air pollution levels. If PAH levels are higher at this time and humans are exposed this could account for the significantly higher levels of AHH activity seen in cultured lymphocytes from students on the Loma Linda University, Riverside Campus at the fall sampling. Since students residing at Pacific Union College are exposed to a low air pollution environment without seasonal fluctuations one might expect to see little or no variation in mean levels of AHH activity as shown in Table I. Students at Pacific Union College who were exposed to a high smog environment during the last 4 weeks of summer vacation and students at Loma Linda University, Riverside Campus, who were exposed to a low smog environment during the last 4 weeks of summer vacation are included in Group B of the fall data along with students who had been sick or were on medication. The number of students in the fall sampling at each campus that were placed in Group B solely on the basis of summer residence was too small to permit a valid comparison of AHH activity with all samples assayed (Group A). Most of these students were also sick and/or on medication. Fewer than 10 percent were smoking.

The values of AHH activity indicated in this report are several-fold higher than those given for cultured lymphocytes previously isolated by sedimentation on Ficoll-Hypaque gradients (17) but compare favorably with values obtained from induced monocytes (18). Different quantitation procedures make it difficult to make comparisons however. This report describes data obtained on AHH activity in cells in the buffy coat which would include lymphocytes and monocytes. This could account for the higher values obtained and for the greater variability. It seems unlikely that genetic factors would account for the differences in AHH activity observed in students from the two campuses since the sample size in each case was large.

A number of factors are known to affect AHH activity. These include cell density, storage of blood, exposure to mitogens, variations in culture medium, serum and culture conditions (19). It is possible that some of the conditions listed above could account for the variability in this study. This is particularly true for the starting cell density and blood storage conditions. Studies indicate a 50 percent reduction in AHH activity in lymphocytes isolated from whole blood stored for 24 hrs (19). Blood collected at Pacific Union College was in storage from 4 to 6 hrs longer because of the transit time and this may be a significant factor contributing to the lower mean values of AHH activity in student lymphocyte samples from this campus. The quantity of cells put in culture differed from sample to sample but the large number of samples employed should negate this as being a significant variable. The same lots of serum, culture medium, and mitogens were used in this study so that variations caused by different lots of these materials should be minimal.

Aryl hydrocarbon hydroxylase metabolizes polycyclic aromatic hydrocarbons and is a component of the mixed-function oxygenases which metabolize steroid hormones, drugs and insecticides (17). Although cigarette smoke is known to contain benzo(a)pyrene attempts to measure differences in levels of AHH activity between smokers and nonsmokers has been inconclusive. Since AHH can be induced by a variety of chemical agents, information concerning the habits and background of each individual is critical to any evaluation of the effects of environmental factors on AHH activity.

The data reported here suggest that environmental exposure to air pollutants induces AHH activity in cultured lymphocytes. Other studies are essential to a determination of the validity of this conclusion. The most reliable and least variable approach would be to measure AHH activity in cultured monocytes (16,18) and correlate AHH activity with ambient levels of polycyclic aromatic hydrocarbons (i.e. benzo(a)pyrene). These kinds of studies could be useful for evaluating the role of air pollutants (PAH) in the carcinogenic process.

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APPFNDIX

Individual Data - Pacific Union College Spring 1977

Activity Units/mg Protein	.62	0.		.91	.34	71	.25	0	.72	.02	.45	.28	.09	0.552	.28	.00	.75	0	.72	.17	96.	5.057	.57	.09	.95	.75	.13	.87	8	
AHH Units	. 59	.37	0.373	.37	.14	.14	.37	0	.82	. 59	.82	.03	.59	0.037	.37	49	.70		.72	. 26	.82	0.708	. 26	.04	.70	. 14	.82	. 14	14	
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AHH Units	14	.48		.59	59	.26	.59	.03	.03	.26	,14	,37	0	∞	.48	.82	. 14	.26	0:149	.26	.48	. 26	.70	. 59	.37	0	:37		.48	
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Individual Data-Pacific Union College (Continued)

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Activity Units/mg Protein	4.550 2.923 2.923 0.00 7.780 7.7	•
AHH Units	0.596 0.149 0.149 0.596 0.708 0.708 0.373 0.037 0.037 0.037 0.037 0.373 0.373 0.373 0.373 0.373 0.373 0.485 0.373	•
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Individual Data-Pacific Union College (Continued)

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	corrected Fluorescence Reading	4				2	. 2		m	2.5		0	∞	9	5.5	6	3.5	2	2.5	2	4		m	2	Ö	0	ĸ	6.5		4.5			or had been	tour weeks prior to
F	lotal Protein (mg)	.127	960	.116	5	.071	∞		9	.065	9	.082	.108	.113	.094	$\overline{}$.122	∞	.90	0	,152	\sim	.084	_	.037	9	_		.093				t was	during the to
	sample No.	6794	6 7 9 8	6814	6840a	6850	6852	6856	858	6859a	863	6 868	897	899	6900a	901	902	903	905	907	908	910	913	914	6915a	916	6917	6935a	6950	6951		ď	Stı	np
	Activity Units/mg Protein	•	2,865	0	0,804	0	2.122	4		0	3,360	4.288	0	0	5.706		0		.84	,48	7	45	.78	99,	0.402	.43	0	.08	3	, 36	10	16.062	.32	
	Uni ts	•	0.149		0.037	0	, 26	0.596	.26	\circ	0.37.3	5	0	0	.48	0.373	0	<u>.</u> 1	14	14	,37	.82	.37	, 14	0.037	. 26	0	.48	.37	4	.93	•	37	
	corrected Fluoréscence Reading	7.5			0.5		٦,٠		٦,5		2	m	0		2.5		0			<u>.</u>	5		6.5		0.5	۰		2.5	2		4.5	آ	2	
;- H	lotal Protein (mg)	0.59	.052	101	.046	.030	.123	.093	,062	.083		.139	.136	,104	.085	.112	.063	690°	.019	090°	.114	.097	.117	.056	.092	,076	,153	.157	191	.077	.077	.065	.07	
r	Sample No.	6664	6668a	1299	6 6 2 9	6683	6685a	6688	6 6 9 1	6693	6705a	6707	713	6714a	720	721	6724	725	726	6727	6730	6733	6734	6738a	6741	6744	6749	1979	6775a	6777	6779	0629	6792	

Individual Data - Loma Linda University, Riverside Campus Spring 1977

Activity Units/mg Protein	9.181 3.391 6.754 9.976 8.138 8.138 12.000 12.000 12.140 7.450 10.543 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314 7.314	
AHH Units	1.267 0.373 0.373 0.708 0.708 0.708 0.708 0.708 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373	
Corrected Fluorescence Reading		
Total Protein (mg)	.138 .138 .127 .087 .087 .059 .059 .059 .051 .074 .073 .056 .051	
Sample No.	5073a 5074a 5074a 5077a 5077a 5080a 5084 5084 5087 5091a 5091 5104 5117 5117a 5117a 5117a 5117a 5117a 5117a 5117a 5117a 5117a	
Activity Units/mg Protein	13.016 3.806 4.287 4.722 1.620	
AHH Units	0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.596 0.596 0.596 0.596	
Corrected Fluorescence Reading		
Total Protein (mg)		
Sample No.	5005 5007 50007 50007 50008 5011 5027 5029 5032 5032 5059 5059 5059 5059 5059	

Individual Data-LLU, Riverside Campus (Continued)

Activity Units/mg Protein	2.289 8.981 2.088 5.623 6.661 1.231 1.231 7.224 7.321 7.321 1.23 7.424 7.698 8.289 3.480 0.755 18.643 0.607	•
AHH Units	0.261 0.261 0.261 0.261 0.373	.37
Corrected Fluorescence Reading	- 01 - 62 - 63 - 63 - 63 - 63 - 63 - 63 - 63	
Total Protein (mg)	100.00.00.00.00.00.00.00.00.00.00.00.00.	.03
Sample No.	5276a 5283 5283 5287 5287 5290 5301a 5307 5317 5317 5318 5320 5320 5333 5336 5336 5336 5336 5336 5336 533	422
Activity Units/mg Protein	2.747 4.139 12.769 11.212 4.440 10.933 12.189 7.532 14.337 14.337 14.337 10.361 10.242 17.356 17.356 17.356 17.356 17.356 17.356 17.356 17.356 17.356 17.356	16,955
AHH Units	0.261 1.379 1.379 0.373 0.373 0.373 0.373 0.373 1.379 0.373 0.373 0.373 0.373 0.373 0.485 0.820 0.485	.37
Corrected Fluorescence Reading	- w o o o o o o o o o o o o o o o o o o	. 2
Total Protein (mg)		.022
Sample No.	5184 51854 51856 5186 5186 52186 5204 5220 52231 52231 52236 52236 52236 52503 52503 52503 52503 52503 52503	5273a

Individual Data-LLU, Riverside Campus (Continued)

		ne.
Activity Units/mg Protein	0.481 9.049 1.088 2.483 7.181 3.943 9.327 16.735 1.795 1.795 10.643	tion sometime d drawing.
AHH Units	0.037 0.932 0.037 0.149 0.596 0.485 0.708 0.708 0.708 0.261	n on medication to the blood dra
Corrected Fluorescence Reading	0.40 8.25.42 8.50 8.50 8.50 8.50 8.50 8.50 8.50 8.50	weeks prior
Total Protein (mg)	.077 .034 .034 .065 .083 .059 .089 .089 .089 .056	Student was sick during the four
Sample No.	5539a 5541 5541 5542a 55443a 55443a 555473 5556 6206 6238	Studen
Activity Units/mg Protein	000000000000000000000000000000000000000	19.930 4.579 9.699 4.440 11.841 6.217 13.127 9.327 14.398 6.428 9.385
AHH Units	40040000400000	1.714 0.261 0.373 1.267 0.373 0.373 0.149 0.149 0.261 1.267
Corrected Fluorescence Reading	22	& - & 4 0 4 0 - 4 0 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
Total Protein (mg)	.09 .037 .080 .058 .058 .051 .077 .035 .035	.086 .057 .057 .084 .052 .089 .035 .079 .145
Sample No.	5426a 5427a 5437 5433 5434 5485 5486 5486 5506 5512 5512	5517 5519 5520 55224 55226 5525 5531 a 5534 5534 5537a

Individual Data - Pacific Union College Fall 1977

Activity Units/mg Protein	4.210 4.287 2.292 6.274 4.605 4.605 0 0.561 6.082 3.527 3.107 2.868 6.808 14.449 1.795 6.808 1.795 6.370 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1.797 1.795 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
AHH Units	0.26 0.373 0.156 0.373 0.373 0.373 0.596 0.596 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.261 0.373 0.261 0.261 0.373 0.261 0.261 0.373
Corrected Fluorescence Reading	
Total Protein (mg)	.062 .087 .095 .095 .096 .096 .096 .097 .091 .091 .091 .091 .091 .091 .091 .091
Sample No.	7236 7239 7244 7244 72444 72444 7254 7324 7324 7324 7324 7324 7429 7429 7429 7429 7437 7437 7437 7437 7437 7437 7437 743
Activity Units/mg Protein	7.95 11.714 11.245 8.394 19.327 2.759 19.153 6.308 8.638 8.652 7.314 8.394 10.380 9.030 5.052 6.214 4.027 0.649 5.181 3.768 10.965 0.425
AHH Units	0.596 0.596 0.596 0.596 0.149 0.149 0.15 0.15 0.15 0.15 0.15 0.15 0.15 0.15
Corrected Fluorescence Reading	w4ww49-04-00-00-00-00-00-00-00-00-00-00-00-00-
Total Protein (mg)	075 070 070 070 075 072 072 072 073 073 073
Sample No.	7004 7086 7087 7096 7101 7104 7110 7112a 7112a 7124a 7124a 7124a 71243 7125a 7147a 7147a 7147a 7155a 7165a 7165a 7165a 7165a 7165a 71701 7187 7187 7188 7187 7188 7187 7188 7187 7188 7187 7

Individual Data-Pacific Union College (Continued)

	,																																
Activitv	Units/mg Protein	.46	9.816	.09	.90	. 75		0		.42	.56	91.	. 28	3.104	. 26		.14	.50	5	3,725	.09	.82	,56	.27	99.	0	.58	2.922		,74	4.806	.27	• 73
АНН	Units	.03	0.373	.37	.37	14	.37	0	0	.82	,37	.59	.37		.70	0	.48	.03	.14	0.149	.14	,37	.37	.48	. 26	0	, 26	.14	0.149	.37	. 14	.59	. 14
Corrected	Fluorescence Reading	. 5.0	2 2		. 2		2	0	0 -	4	2	n	2		3,5	0	2.5	. 0.5				2	. 5	2.5	7.5	0	1.5			m		ω ,	
Total	Protein (mg)	070	.038	.091	.054	.054	.027	.022	.033	.087	.039	.049	.045	.048	690°	.062	.117	,074	.027	.040	.071	.064	290.	.093	,056	.039	.101	.051	.142	.077	.031	. 13	• 026
Sample	No.	693	7705a	7707	714	7721a	7724	7726	7727	7730	7734	.7738	7741	744	7750a	1977	7775	7777	7790	7792	794	798	7840a	856	858	7863	7868	899	7900a	902	7903a	905	/9084
Activity	Units/mg Protein	0	3.548	\circ	9.1	7(3)		(٢)	נא	ΓŪ	0	ι.	9	Q.	α	۲.	·	S	4.	٠٣٠	9,	ις.	4	o.	4	က္	0	0		.03	•	\sim	.86
ДНН	Units	, <u>6</u>	0,149	1.379	.48			4.	•	∞	. 2	<u> </u>	_	4	2.	ς.	₽.	3	.5	0.267	0.	3	0.	4.	.	$^{\circ}$	0	0	.03	59	•		_
Corrected	Fluorescence Reading			. •	2.5	۰	9.	2.5		4	2.5			2.5	7.5	5	2.5	2	က	1.5	0.5	. 2	0.5	7	2	2	0	0	0.5	 		5.	
Total	Protein (mg)	.075	.042	.051	.053	.047	960	160.	.095	.078	.052	.059	032	.061	.054	.078	.040	.115	080	.040	. 059	.057	.077	660.	690	. 036	.038	.041	.053	.054		.053	
Sample	No.	7493ª	7506	7516	7519	7521a	7543a	7545	7547	7549	7550	7557	7558	7568a	7578	7587	7588	7602a	7604	7607a	7614	7619a	7620	638	7643a.	7650	7664	7668ª	7671	7679a	76,83a	85	

Individual Data-Pacific Union College (Continued)

Activity Units/mg Protein										
AHH Units										
Corrected Fluorescence Reading										
Total Protein (mg)										
Sample No.	•									
Activity 'Units/mg Protein	4.292	4.663	0	2.056	5,254	0.841	0	4,722	12,763	
AHH Units	0.485	0.373	·0	0.037	0,373	0,037	, 0	0.373	0.485	
) ce							٠	٠.		
Corrected Fluorescence Reading	2.5	2	0.5	0.5	. 2	0.5	0	2	2.5	
Total Protein (mg)	. 13	080	090	.018	1.70	.044	.040	.071	.038	
Sample No.	7910	7914ª	7915	7917	8184	8519	8506	8219	8072	

Student was sick, had been on medication sometime during the four weeks prior to the blood drawing, or had spent the last four weeks of the summer vacation in a high smog environment.

Individual Data - Loma Linda University, Riverside Campus Fall 1977

Activity Units/mg Protein	8.289 12.681 19.914 5.000 7.000 11.548 2.000 4.766 4.766 10.581 10.581 12.125 12.125 12.125 13.558 11.303 3.634 23.463 23.463	
AHH Units	0.373 0.596 1.155 0.485 0.261 0.261 0.261 0.261 0.261 0.335 0.335 0.335 0.335 0.335 0.373 0.373 0.373 0.596 0.373 0.596 0.373 0.596 0.596 0.373 0.596 0.596	
Corrected Fluorescence Reading	2000 4 8 8 9 7 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	
Total Protein (mg)	0045 0045 0045 0045 0047 0040 0040 0040	
Sample No.	8082 8084 8087a 8087a 8091 8091 8093 8108 81174 81174 81174 81175 81170 81170 81170 81170 81170 81180	
Activity Units/mg Protein	9.440 7.771 8.343 7.460 6.016 3.386 10.591 6.016 1.000 12.960 17.029 13.050 13.050 13.050 15.533 18.644 10.242 11.081 7.823 15.533 18.644 10.242 11.081 7.823 15.533 15.533 15.533 15.533 17.656	
AHH Units	0.708 0.596 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.444 0.485 0.932 0.933 0.932	
Corrected Fluorescence Reading	wwxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx	
Total Protein (mg)	075 0075 0040 0060 0060 0075 0075 0075 0075 0075 007	
Sample No.	8005a 8005a 8006 8008 8008 8011 8011 8011 8011 8011	

Individual Data-LLU, Riverside Campus (Continued)

Activity Units/mg Protein	4.782 11.095 7.069 23.095 5.008 3.104 5.300 1.568 9.598 8.220 3.926 10.356 6.525 14.346 2.709 13.050 22.242 1.935 7.612 10.235 17.356	.02
AHH Units	0.373 0.820 0.820 0.820 0.149 0.149 0.335 0.335 0.335 0.344 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.373 1.044	48 48
Corrected Fluorescence Reading	244281871891841841841841841841841841841841841841841	2.5
Total Protein (mg)	.078 .078 .021 .035 .095 .095 .036 .039 .049 .049 .037	.044
Sample No.	8299 8301a 8304a 8307 8310a 8317 8320 8321 8320 8329 8329 8329 8329 8329 8329 8329 8329	8434a 8445
Activity Units/mg Protein	7.771 7.771 3.170 17.205 17.205 17.205 17.205 17.205 17.695 1	.84
AHH Units	0.373 0.373 0.373 0.373 0.373 0.373 0.373 0.320 0.335 0.335 0.337 0.335 0.335 0.337 0.373	.26
Corrected Fluorescence Reading	2.22 1.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2	2 - 2
Total Protein (mq)	042 0042 0057 0053 0053 0053 0053 0053 0053 0053	.015
Sample No.	8210a 82110a 82110a 822114a 82220 82231 82235 82235 82250a 82250a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a 82550a	90

AIR POLLUTION QUALITY CONTROL DATA

The pertinent APA data is being processed at Research Triangle Park and will be made available after February 28. It is therefore not possible to include the air quality control data from each college campus at this time. It will be added when it is made available.