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EFFECTS OF INHALATION OF FREON 113 ON LABORATORY ANIMALS

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Freon 113 (1, 1, 2-trichloro 1, 2, 2-trifluoroethane) is one of the recent ethanes to find wide industrial application as a refrigerant, solvent, and cleaning agent. Toxicity studies have shown it to be relatively nontoxic, and the American Conference of Governmental Industrial Hygienists[1] has assigned a Threshold Limit Value (TLV) of 1000 ppm. The primary response to exposure appears to be alteration in central nervous system function with concentrations of 2500 ppm and above inducing deterioration in psychomotor performance in humans (Stopps, 1967).

This presentation will cover the results of two recently completed studies on the toxicology and pharmacology of Freon 113. The first study was designed to determine the biological effect level for inhaled Freon 113 under continuous exposure conditions, for subsequent long-term experiments. The exposure concentration chosen was 2000 ppm for a period of 14 days. The second study was designed to determine the effects

[1]Threshold Limit Values of Airborn Contaminants Adopted by ACGIH for 1970.

of Freon 113 on transmission through autonomic ganglia. The experimental method selected for this study utilized the stellate ganglion of the spinal dog with the increase in heart rate during pre- or postganglionic stimulation serving as the end organ response. This preparation was utilized by Garfield et al (1968) for determining the degree of nicotinic and muscarinic blockade induced by certain anesthetics. It was selected by us in an attempt to compare the efficacy of Freon 113 as a ganglionic blocking agent with that observed by halothane.

METHODS

14 Day Exposure

The experimental and control groups of animals were comprised of 4 monkeys and 8 dogs in each group; 40 mice and 50 rats were used in experimental groups, and control groups consisted of 20 mice and 25 rats. Monkeys and dogs were females while rats and mice were males. The exposure was conducted in a Thomas Dome at ambient conditions.

The "Freon 113 TF" used in this study was purchased from the E. I. DuPont De Nemours Company, Inc., Wilmington, Delaware. Freon 113 contaminant analysis was made by gas chromatography as described by MacEwen and Vernot (1970).

Ganglionic Study

Female beagle dogs weighing from 8.0-13.7 kg were used in this study. The methods for preparing the animals were the same as those described by Garfield et al (1968). Briefly, they consisted of anesthetizing the dog with thiopental sodium (approximately 30 mg/kg intravenously), ligating both carotid arteries and severing both vagosympathetic trunks in the midcervical region, severing the spinal cord at the atlanto-occipital junction, and destroying the brain stem with a probe. This procedure eliminated the necessity for any further drug anesthesia. The chest was then opened with a midline incision, the right stellate ganglion isolated, and stainless steel electrodes placed on the pre- and postganglionic nerves to and from the ganglion. The heart rate was recorded from pericardial electrodes. Arterial pressure and arterial samples for blood gas analysis were obtained through a catheter placed in the femoral artery. A Statham model P23 pressure transducer was used to measure aortic blood pressure which was recorded with the heart rate on a Grass model 5D polygraph. Mean arterial pressure was maintained above 50 mm Hg by intravenous infusion of 6% dextran as needed.

Ventilation with 100% oxygen was maintained with a Havard respiration pump. Rate and tidal volume were adjusted to maintain arterial blood oxygen partial pressure at 500 mm Hg or above. The 2% Freon 113 was prepared by vaporizing a predetermined amount of the Freon into a set oxygen flow and delivering the mixture into a reservoir. The contents of the reservoir were then delivered to the dog by the Harvard pump. The reservoir mixture was periodically checked by gas chromatography to assure that a 2% mixture was actually being delivered to the dog.

Square wave stimuli of 1 msec duration were delivered to the nerves by a Grass model S4 stimulator through a stimulus isolation unit. The experimental procedures followed were the same as those described by Garfield et al (1968) with one major exception. After supramaximal voltages had been determined, control increases in heart rate were recorded following stimulation at 1 and 3 cps for each of the pre- and postganglionic nerves. Inhalation of 2% Freon 113 was then initiated. Ten minutes was then allowed to elapse and the heart rate response to both pre- and postganglionic stimulation at all frequency ranges was determined (0.3 to 40.0 cps preganglionic and 0.1 to 10.0 cps postganglionic). The Freon 113 exposure was then terminated, 15 minutes allowed to elapse, and control heart rate responses to both pre- and postganglionic stimulation determined. This procedure of obtaining the control observations after the exposure prevented the possibility of confusing the action of the test compound with that reduction in response observed in a deteriorating preparation.

A three factorial analysis of variance was used to determine the statistical significance at the 5% level between the control and exposure observations as well as between the exposure and exposure plus atropine observations.

RESULTS AND DISCUSSION

14 Day Exposure

The changes observed in animals exposed to 2000 ppm of Freon 113 for 14 days were all minimal, and could not be related to the toxic effects of the compound. Enlarged thyroid glands were observed in all rhesus monkeys exposed. Rat kidneys were the only organs showing an increase in weight over control values. These differences were minimal, and could not be conclusively attributed to the exposure.

Ganglionic Study

The ganglionic effects produced by inhalation of 2% Freon 113 in 4 dogs are seen in figures 1 and 2. Two percent Freon 113 significantly reduced the increase in heart rate produced by preganglionic stimulation at all frequencies from 0.3 to 4.0 cps. The exposure had no effect on the heart rate response to postganglionic stimulation. Further reduction in the response to preganglionic stimulation was seen at stimulation frequencies of 1, 3, 6, 10 and 40 cps by the administration of atropine, 0.05 mg/kg intravenously. This increase in the depression by muscarinic blockage during Freon 113 inhalation could be indicative of nicotinic blockage in the stellate ganglion.

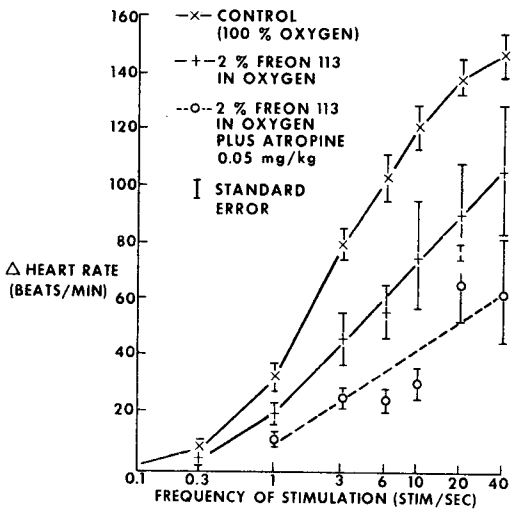

Figure 1. THE EFFECT OF 2% FREON 113 ON PREGANGLIONIC STIMULATION

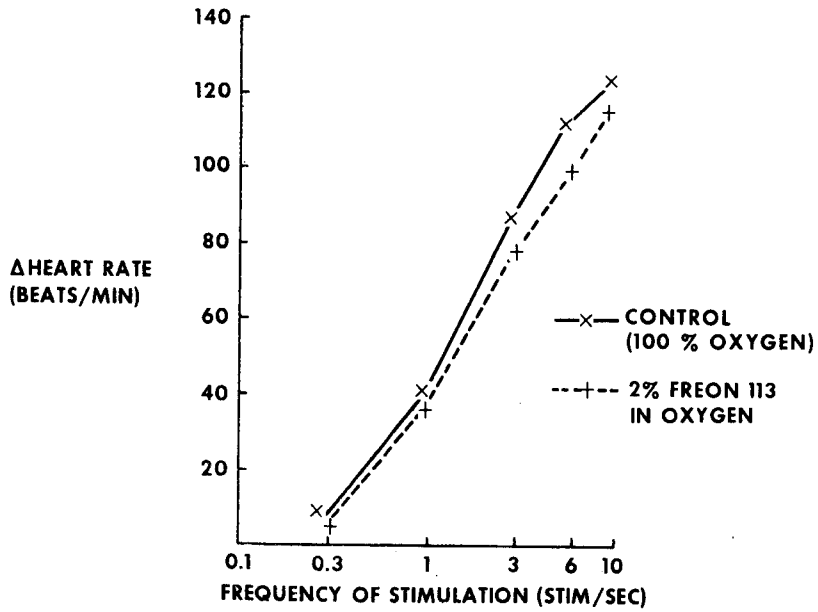

Figure 2. THE EFFECT OF 2% FREON 113 ON POSTGANGLIONIC STIMULATION

Garfield et al (1968) utilized this preparation to determine the effect of certain anesthetics on nicotinic and muscarinic transmission through autonomic ganglia. They found that halothane, among other anesthetics disrupted both classes of cholinergic, ganglionic transmission. The degree of nicotinic blockade produced by 2% halothane as demonstrated by these investigators is presented in figure 3. Although comparing data of this type between laboratories is very difficult, it does appear that the nicotinic blockade produced by 2% halothane and 2% Freon 113 are comparable.

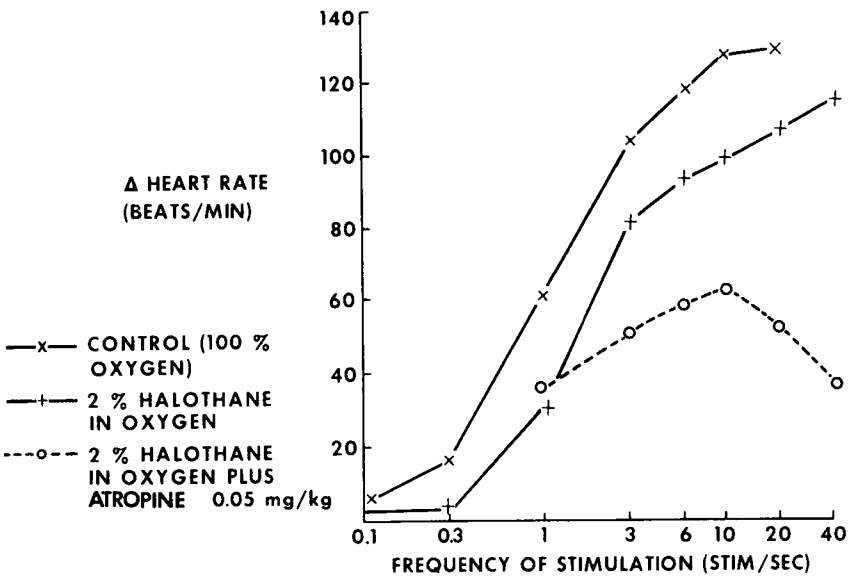

Figure 3. THE EFFECT OF 2% HALOTHANE ON PREGANGLIONIC STIMULATION

*Taken from Garfield, J.M., Alper, M.H., Gillis, R.A. and Flacke, W. (1968). A Pharmacological Analysis of Ganglionic Actions of Some General Anesthetics. Anesthesiology 29, 79-92, and used with the author's permission.

The results of Garfield et al (1968) suggest the possibility of a relationship between the ability of certain anesthetics to antagonize both nicotinic and muscarinic transmission in sympathetic ganglia and their efficacy as anesthetics. Burn (1959) has shown that approximately 5 times the concentration of Freon 113 compared to halothane is required to produce anesthesia in mice. Our data indicate that Freon 113 and halothane in equal concentrations have approximately equal efficacy in reducing nicotinic transmission through the stellate ganglion of the spinal dog. Unanswered is the ability of Freon 113 to reduce muscarinic transmission through the ganglion, and if so, at what concentrations. It is entirely possible that anesthetic concentrations of Freon 113 would be required to antagonize muscarinic transmission. If so, this may help explain the large difference between the concentrations required to produce initial central nervous system depression and that required to produce anesthesia.

SUMMARY

Four monkeys, 8 dogs, 40 mice and 50 rats were exposed continuously to 2000 ppm Freon 113 in a Thomas Dome for 14 days. This exposure produced no mortalities nor adverse symptomatology. There were no significant alterations in hematological values, clinical chemistries, electroencephalographic findings, body weights, or organ to body weight ratios. The effect of 2% Freon 113 on nicotinic transmission through the stellate ganglion of the spinal dog was also evaluated. This exposure induced a reduction in nicotinic transmission comparable to 2% halothane. The effects of this compound on muscarinic ganglionic transmission were not evaluated.

REFERENCES

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3. MacEwen, J.D. and E.H. Vernot; "Toxic Hazards Research Unit Annual Technical Report"; AMRL-TR-70-77, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, 1970.

4. Stopps, G.J. and M. McLaughlin; "Psychophysiological Testing of Human Subjects Exposed to Solvent Vapors"; Am. Ind. Hyg. Assoc. J., 28, 43-50, 1967.

DISCUSSION

DR. MAC EWEN: As I remember from reading reports and hearing about Freon 113 at meetings, it is essentially non-metabolized or only slightly metabolized and is excreted by the lung very rapidly after exposure. Do you have any information on the levels remaining? Did you do any breath analyses postexposure?

MAJOR CARTER (NASA, Manned Spacecraft Center): No, we didn't. We do have some data from Dr. Kaplan. He did some exposures in the dome and there is the slide. The last slide in the series, and I can't really comment on it, except that these were the data that Dr. Back gave me that he found, with 20 rats exposed. At the end of seven days, we sacrificed 5; at the end of 14 days, we sacrificed 5 more; then 24 hours post-exposure, 5 more, and then 48 hours. Notice that there is no real difference between the 7 and 14 day exposure level, except for some reason in the adrenals he gets less Freon 113 at the end of 14 days than he does at 7. I suppose if he were here he would like to comment on that, I don't know what it means. Then at the end of 24 hours post-exposure you do find some in fat, although it's dropped about 85% and at 48 hours, there is less than 1% of the original level. After 24 hours there is none in any of the other tissues that he tested.

TISSUE	EXPOSURE		POSTEXPOSURE	
	7 DAY	14 DAY	24 HOURS	48 HOURS
BRAIN ug/gm	22.73 (1.00)	22.65 (1.33)	NONE	NONE
LIVER ug/gm	15.77 (0.87)	16.40 (1.72)	NONE	NONE
HEART ug/gm	16.59 (2.56)	15.03 (2.51)	NONE	NONE
FAT ug/gm	722.48 (71.29)	659.24 (21.17)	108.45 (33.62)	5.60 (2.94)
ADRENAL ug	8.39 (2.61)	3.47 (0.34)	NONE	NONE
THYROID ug	1.09 (0.46)	0.94 (2.00)	NONE	NONE

( ) STANDARD DEVIATION

MEAN TISSUE CONCENTRATIONS OF FREON 113 FROM RATS EXPOSED TO 2000 PPM

DR. BACK: The reason I can't say a lot about it is because I didn't do anything but help him harvest the tissue. But this is gas chromatography and the replication is really good between the pieces of tissue that he took. Except that he took all of his data with him to Thailand, so I can't do anything but show what we've got here. Now, after the seventh day, he just took things out of the sample bag for analysis, and I did all the cutting myself so I have to take the blame for the adrenal gland, I think that I left too much fat on the adrenal gland. At the fourteenth day, I used a head lamp and I got every bit of the fat off, they were absolutely clean and I think this is why you see the difference. If you notice that the fat does pick it up after a relatively long exposure. We did some cursory experiments at the end of an exposure for 30 minutes, and the fat does not have time to pick it up under those conditions. So it's blown off very, very quickly in the breath so that if animals are exposed for 30 minutes, 10 minutes after exposure it's all gone. This is also true with Freon 1301, but when you do exposures for 14 days, I think you could see that it is picked up pretty well and in fact 24 hours later there is still some in the fat, and 48 hours later there is still a little bit. It is all gone the next time he looks at it.

MAJOR CARTER: Before Colonel Steinberg asks his question, we also did some other exposures in connection with the autonomic ganglia work. We exposed dogs at 1% and there was no effect also exposed some to 4%. Right now I'm trying to have that data compared with 2%. We used the animals as their own controls. It is very difficult to compare four animals here with four animals over there. Our statistician at NASA says he has a method for doing this. I hope he's still there when I get back. But there doesn't really appear to be any difference at 4% compared with 2%, as far as transmission through the nicotinic portion of the autonomic ganglia is concerned.

COLONEL STEINBERG: The second question I was going to ask you: this didn't look like a complete blockade, so you would expect to get a little more blockage with increase in the dose, but one question, this is an open chest dog you stimulated?

MAJOR CARTER: Right.

COLONEL STEINBERG: Well, one thing I didn't notice, maybe this is on the slide, is after you've blocked the heart and vagotomized it in an open chest preparation you would expect that when you did do postganglionic stimulation, you should have gotten a greater response than you got in your initial controls, because you should have a sensitive preparation in terms of stimulation.

MAJOR CARTER: Right. You mean the effect of the compound should cause a potentiation of the postganglionic response?

COLONEL STEINBERG: Right.

MAJOR CARTER: Because of the sensitization of the myocardium? We didn't see this. It has been seen with Halothane. At 0. 25% with Halothane, and postganglionic stimulation, you do get a larger response than you do with no Halothane. Now, at 1% you're getting enough blockade that you're essentially seeing nothing. You've potentiated your response, as far as the heart goes, and you have a small degree of ganglionic blockage but the thing is controlled when you do stimulate postganglionically. You will see a slight potentiation. Now, I think maybe we might have seen this if we had had a pure postganglionic nerve. But in only one dog did we get what we considered a pure postganglionic nerve. In one animal we had after C-6 we would get about a 30% drop in heart rate increase from stimulation of supposedly postganglionic nerve, which would indicate that there are some preganglionic fibers there. I don't know why, we're putting the electrodes the same place, supposedly that the other people were, so I have no explanation. I can't really say as to whether we were getting some potentiation there or not. Does that answer your question?

COLONEL STEINBERG: Yes. If that was an open chest since that's one of the ways used to simulate heart failure, do you think this had an effect? What did your EKG show?

MAJOR CARTER: The EKG was quite rapid. The baseline rate runs around 120 to 150. Now, you're driving it so that you get a delta heart rate of around 150, that means that you're driving the heart around 300 and I was quite surprised because I thought we would see some PVC but we didn't. And this makes one wonder what causes the PVC. One would think that you would get enough epinephrine released from the nerve, if this is contributing to the PVC's that you are supposed to see with these halogenated compounds. Now I notice in the literature, Garfield and coworkers, they didn't see any PVC's either with Halothane. So I don't know. Dr. Back, I think, has a comment.

DR. BACK: I think there are two things you've got to look at here from some work that Major Van Stee has done. One of them is that you're working against yourself because the compound probably does cause a decrease in diastolic pressures, and that the heart is not capable of pumping that hard, maybe. So, you've got that going against you, and also I think it has been shown with 1301, which probably can carry over to this compound, that you've got a change in total peripheral resistance. In this preparation you've got a 50 mm blood pressure, now Major Van Stee showed that it could mechanically increase blood pressure and hence increase cardiac irritability. In other words, mechanically he could titrate an animal in and out of fibrillation merely by giving more blood or taking away blood, hence increasing or decreasing blood pressure. So that as he dropped the blood pressure, the fibrillation spontaneously quit. Now with a 50 mm blood pressure maybe this is another compensatory thing that won't allow it to do it. What do you think about that, Major Van Stee?

MAJOR VAN STEE (Aerospace Medical Research Laboratory): I think that the hypotensive state of this animal probably provides the answer to the absence of PVC because in a similar way to cyclopropane, epinephrine induced arrythmias and Halothane induced arrythmias are sensitive to blood pressure, but I would be reluctant to suggest a mechanism by which this was brought on. One may mechanically alter blood pressure

by a number of ways; by expansion-reduction of the circulating blood volume; by alternately constricting and releasing the aorta, methods of this type, and control the appearance of PVC's. And since we've been working with animals which were hypotensive and perhaps uncompensated heart failure too, I would guess, they probably did have an elevated left ventricular and diastolic pressure. This is probably why you didn't see any PVC's. Circulating catecholamines decrease blood pressure threshold required to trigger arrythmias, as in the case of 1301, but I would guess that down around 50 or 60 torr mean blood pressure that you probably would not have a high enough blood pressure to trigger arrythmias.

MR. STEVENS (Ohio State University): Major Carter, do you attempt to antagonize any of the effect of some of these compounds?

MAJOR CARTER: Did I attempt to antagonize the blockage that I saw, I'm sorry I don't understand the question.

MR. STEVENS: Did you attempt, with other compounds, to block the effect of some of these Freons?

MAJOR CARTER: No. Only the administration of atropine to cause a further shift in the curve to demonstrate that it was a nicotinic blockade. Now, one could ask, does the compound possibly have any muscarinic blocking properties? In this prep, which is seen also with others, when Eckart first demonstrated in some autonomic ganglia that there was a muscarinic compound, atropine alone does not cause a shift in the curve. To demonstrate muscarinic blockade one must first give the C-6 and then administer the compound. If you get a further shift in the curve this would indicate muscarinic blockade. We didn't do this mainly because I didn't have time, I had to leave, Dr. Chikos had to leave. I would say that, since Halothane has no muscarinic blocking properties and any of the compounds along this line seem not to, that this compound wouldn't either. I think that the blockade that we see is pure nicotinic blockade. But I can't say for sure.

DR. JACOBSON: Are there other questions? If we exhausted Freon 113 and Major Carter, both, would you care to explore Air Quality Criteria any further?

MR. ADAMS: Dr. Hueter, can you give us your definition for total hydrocarbons? I've always been confused by this when I've seen it in the literature. What are your ideas on this?

DR. HUETER: The criteria document that has been published, I believe entitled "Reactive Hydrocarbons", was intended to respond to reactive hydrocarbons and the need for the criteria document was not intended to be a health effect need. It was intended to be a need so we could tie the package together for photochemistry. Whereby, you can control hydrocarbon, and say NO_X and thereby end up with something that also controls oxidants. You can't consider one of these without considering the other two. So, of course, as total hydrocarbons are generally measured, they're expressed as

methane. Other than having some important relation to photochemistry, I don't think they have any definitive meaning in terms of toxicology. You have to measure the individual compound if you're talking about toxicology. I don't know if I've answered your question, I tried to explain really what was done, what was the intent of being done. That was one reason why the polynuclear carcinogens were not included in that document.

DR. SCHEEL: Dr. Hueter, I think the question was intended to ascertain whether there was any differentiation between ketones and aldehydes, as opposed to flat-out hydrocarbons. As I understand the total hydrocarbon, this is an all-inclusive term which simply means all burnable carbons.

DR. HUETER: That's what I meant by saying it's an expression of hydrocarbons in terms of methane. So I'm in complete agreement with what you just said.

DR. MAC EWEN: I'd like to go one step further. I know it's not fair to ask you to comment on the paper given by Mr. Stevens earlier and expect you to answer. But in his slides he showed that the gas chromatographic separations, measurements of the total hydrocarbon and methane, methane being a direct measurement did not include the hydrocarbons. The bar graphs showed the methane portion of it to be almost 95% of the total hydrocarbons. Could you expect this to be representative of raw gasoline or refinery wastes? There would be very little left if 95% is methane.

DR. HUETER: I'm really not an atmospheric chemist or air quality man either, I would only say that it is in Los Angeles, for example, the product of primary automobile exhaust and I believe that Mr. Stevens did say that most other hydrocarbons, other than methane are reactive hydrocarbons and have input into photochemistry.

DR. MAC EWEN: The reason I comment is that methane was a direct measurement and the other total hydrocarbons were expressed as methane so that the difference could be a real difference. I'm not sure of the absolute numbers but the percentages showed very little difference.

DR. HUETER: Yes, I believe you're right, but I think that the one point he was making was that it is NAPCA's feeling that we must have a method of differentiating between methane and other hydrocarbons in order to effectively institute any kind of meaningful control procedures.

MR. TOLIVER: When they speak of total hydrocarbons are they speaking only of compounds particularly of hydrogen and carbon, and no other heteroatom, or any carbon bearing compound that's basically organic?

DR. HUETER: I can answer the question by asking a question. Since I am not a chemist, the methodology for measuring the total hydrocarbon is flame ionization, whatever that measures. Will that answer your question?

MR. TOLIVER: That was the reason for my question because your total hydrocarbon can be quite deceptive if it is measured by flame ionization because you detect the response to heteroatoms, that is other than carbon for instance, oxygen, sulphur give you a negative result and what one gets is an additive result of the entire species present. So it is possible to have other chemical species present in appreciable concentrations that will reduce significantly what you're seeing.

DR. HUETER: I think it's obvious also, that NAPCA is not happy with their present method of measuring it.

DR. SCHEEL: I'd like to say thank you for putting Mr. Toliver on the program this afternoon because I think now that if he would write up his paper and put it into the proceedings, we will finally get some agreement between gas chromatography in different laboratories. This would be a great assistance to most people, I've found, the information they've covered is so scattered that they don't bother to dig it out, put it together, and I think he did a beautiful job of putting it together for us.

MR. TOLIVER: I wanted someone to ask me a question because one finds when one finishes the paper, you never quite say exactly what you wanted to say. I did want to add this that this particular approach is sometimes called the poor man's mass spectrograph and the reason for this is that one gets a number like 468, which I had on there as ethanol and from this number one can determine that this represents ethanol. So it is a way of identifying compounds and reproducing it from laboratory to laboratory which is a most important thing.

MR. VERNOT: The problem I have found is that retention time whether relative or absolute is a function of many unknown factors.

MR. TOLIVER: Retention time?

MR. VERNOT: Retention, any measure of retention, as a means of identification of materials analyzed gas chromatographically. When you are faced with a reasonably complex mixture, say something like 25 compounds, as an old gas chromatographer you know you're probably not going to get 25 peaks, you're going to get superimposition of peaks. You're not going to get nice, easily characterized compounds, you're going to get polyfunctionality both within any one particular molecule and among the molecules themselves. When you try to use any systematically developed but empirical technique, which is what many of the index techniques are, you soon find that you're lost in a maze of complexity, because trying to run under various conditions, not only would your retention times change, but the number of peaks would change, and you don't know which peak is which when you look at the different charts. Now, it's true when you're dealing with one compound, or with two or maybe with three, this index identification is a fairly simple matter, but as soon as you get into a somewhat complex situation then the whole thing tends to fall apart and this of course is where specifically the mass spectrometer becomes quite important, even though there are other means of identification of the materials. It lets you see what is coming out of the gas chromatograph.

MR. TOLIVER: Thank you, and I'm glad you brought this up. Two problems: coincident peaks, one has to assume, of course, that the primary function of a gas chromatograph is to separate, and under optimum conditions, one can separate ideally as many compounds as possible according to the phenomena going on in the column. However, one can get coincident peaks, but what one finds instead of using one column, unless the compounds are very, very, very much alike, and we are now able as you know, to separate optically actively compounds, which means that they are simply mirror images of each other. There are certain other new areas that we have in column technology that allow us to vary, to separate most of the kinds of compounds that make coincident peaks. So I must say that if we are good gas chromatographers we can analyze 95% of the compounds we come across. Most good analytical techniques don't require that you analyze more than 95%.

MR. VERNOT: I'll keep this short. The fact is, however, that when you have a large number of peaks and you run them under different conditions, you don't know which peaks were what in the original gas chromatogram. You can't extrapolate back from one peak in one gas chromatogram to the same peak in another chromatogram.

MR. TOLIVER: Let's assume that we're talking about, let's say ten compounds. And the ten compounds all come out with ten different retention numbers, right? Now any one or more of these ten compounds have one or more peaks in them, right?

MR. VERNOT: Yes.

MR. TOLIVER: All right fine, if one runs another gas chromatograph and another column, obviously because of the intermolecular forces in there, one should not get the same kind of separation. True?

MR. VERNOT: No, but you don't know which is which.

MR. TOLIVER: But you do come up with a number, there is a possibility of having the same number as one has with almost all other instrumental approaches, you can come up with the same number. But not on the same column, and according to the number of columns you have run, you reduce the likelihood of not being able to identify the compound. Obviously there are some compounds, I'll admit readily that perhaps 5% of the compounds, we cannot separate. This is not a fault of Kovats Indices, this is a fault of gas chromatography.

MR. VERNOT: I'm not sure I can answer that. Let me say something about mass spectrometry, that is that it's true that there are problems as far as mixtures are concerned, but if the mixtures are kept, let's say to four components----

MR. TOLIVER: You said 25.

MR. VERNOT: No, I'm talking about any one gas chromatographic peak now. You have four components in any one gas chromatograph peak, it's not a difficult thing to first find out that you have four components from purely mass spectrometric information and then to analyze what those things are.

MAJOR ARNOLD (Aerospace Medical Research Laboratory): I was just going to ask Ed, how many peaks he would imagine that he is going to get out of this mass spectrograph when he injects a 25 component mixture into the mass spec?

MR. VERNOT: I'm not sure I--you mean mass spectrometric peaks, well, of course, that's how you analyze the mass spectrum. You may or you may not. It depends on how that particular compound or mixtures of compounds break down in the mass spectrometer.

MR. TOLIVER: But one of the difficult problems that we had, when we talked about this particular approach with mass spectroscopists is that they think we're attacking mass spectrometry. I'm not attacking it, I'm simply suggesting an approach that says the more positive information you have on identification, the more likely you are to identify the compound. So I simply suggested another method to identify the compound, and for those people who don't have the money to buy sophisticated and more expensive instruments, this is a way of representing the data in the open literature that can be duplicated.

DR. SCHEEL: I have had access to both these techniques and used them both and I would just simply like to comment that the gas chromatograph does have one advantage over the mass spectrometer, especially when you start talking about cyclic compounds, if they contain more than one ring. The gas chromatograph is able to separate these and give them to you in single peaks, which you can then run into your mass spectrograph. The mass spectrograph breaks down compounds and you first have to find out how they break before you can identify them. Now this particular facet of the gas chromatograph is especially valuable because we very rapidly, in biological systems, run out of straight chain compounds and run into cyclic compounds, which may be heterocyclic or homocyclic, depending upon what we are looking for. The sensitivity in the past years on the gas chromatograph has been increased tremendously. We, in addition, can use isotopic labelled compounds and calibrate specifically. So, I think there are places for both techniques and I think they both have a tremendous use, but the point that we brought out in Mr. Toliver's talk here, was that he has now taken exactly what Vernot was saying was the trouble and begun to put it down in numbers, which become identifications because you run your calibration curve with a known compound for the column. This then allows us to now compare this column with another column and another column and another column and come up with the same answer.

MR. TOLIVER: Thank you for your help, but let me correct you just this much. The numbers are run by a standard referee in London. There are about five or six in the world and the numbers that we get off the instrument are basically not run in terms of standards per se but between the homologous series. It's a small point.

DR. SLONIM (Aerospace Medical Research Laboratory): There is one thing that I didn't understand in your talk. If you had a mixture of compounds, a large mixture and suppose you did have coincidental peaks, how do you know you have more than one compound, if it only shows one peak at that particular retention number?

MR. TOLIVER: All right, let's assume that the molecular structure of the two compounds is different, but because of the hydrocarbon portion of the molecule and the functional portion of the molecule, let's make one an acetone group and the other an alcohol. You've got C = 0 and OH group. On that particular column they both come off at the same place. Now, as you probably know, alcohol is a little more polar than acetone, so when you put it on a little more polar column, the intermolecular forces would cause alcohol to be held longer and you should get a separation. Did I answer your question? I'm saying that if the molecular structure is relatively different that you can separate them on different columns.

DR. SLONIM: But you've had information prior to this, I mean, you already had some idea of the compounds, in fact whether there are polar or non-polar groups here. Let's assume you have a mixture and you do have a couple peaks where instead of four or five compounds as your chromatogram mixture, you may be dealing with about ten compounds. My question is would it be false security assuming that that's all you had, if in fact you do have multiple peaks as a result of the mixture, which sort of supports what Ed Vernot was saying as to whether or not, using your gas chromatograph, unless you knew exactly how much you had in there, you wouldn't even know to go to another column to separate them?

MR. TOLIVER: One normally uses more than one column for this kind of identification. The original work, you will notice, reported a polar and a non-polar column. But always a minimum of two columns of different separation characteristics. Of course, one can go through as many columns as one likes to identify beyond a doubt what the compound is.

DR. SLONIM: You feel that given one retention number from one peak and by using more than one column in time----

MR. TOLIVER: Let's see if I can do this thing on a particular column, let's take Apiezon L since it's probably the most worked column. It is possible to take the number 468 and have more than one compound that has that number, but the likelihood of that compound on another column, those five compounds from another column having the same number are reduced. And the more columns one uses, the more one reduces the probability of that particular number falling at that same spot. That's the same kind of thing that one does in infrared, the same kind of thing that one does in most analytical methods. No difference, absolutely none.

DR. JACOBSON: Are you interested in pursuing this further, Mr. Vernot?

MR. VERNOT: If I gave the impression that I wanted to substitute mass spectrometry for gas chromatography, I certainly didn't mean to do that. Modern analytical chemists, dealing with materials of any vapor pressure at all, cannot do without gas chromatography and we can't do without it here. I'm merely saying that there are presently so many techniques available for the marriage of gas chromatography and mass spectrometry, that if a mass spectrometer is available, one can get this kind of information as far as identification of molecules of interest, perhaps more unambiguously than by Mr. Toliver's method.

DR. MAC EWEN: I have a question I would like to ask Dr. Ingram or rather two questions. The first one is how do you get an even blood smear for use in the cell scan GLOPR?

DR. INGRAM: Well, we have developed a new method for producing smears which is remarkably good, this is not really new, it's only new for smears. It is adapted directly from the technique used for coating small optical parts with photoresist. If I could draw a little picture, I can explain it very quickly. This is a spinner technique that I was using. We use a spinner made by Pratt Engineering Company in Chatham, Pennsylvania. They make it for industrial use and any other purpose. It has a rotor spinner, slide, and once in position a cover glass. It's held on by a powerful suction to the spinner and there is a safety catch that goes on when it spins. It sounds like an outlandish method, but using blood which has been treated with EDTA to prevent coagulation and clumping, the cover glass is placed down here. Then we flood it with the blood which has been mixed so that there is a fairly uniform suspension of cells. You can actually see the top of the meniscus over the whole cover glass. The cover glass rotates and it is designed with a clutch mechanism which can be used so that you reach full speed in about 30 milliseconds; or you can use a slower speed of one second. After that it really doesn't seem to make much difference if you slow it down further. There is a speed regulator. Push the button and it spins rapidly, the blood goes flying off in all directions and you end up with a perfectly beautiful, absolutely uniform smear of blood cells, bone marrow, blood platelet suspensions--what have you.

It's really remarkable, we did a lot of work to document this. We did a lot of cover glass smears, differentials, and cell counts with Professor Garret. The cell distribution over the smear is absolutely random. There's no collection of large cells. At the extreme edge there's a very, very thin rim which is different, but that's negligible.

The thing that's most important about it is that there is almost no disruption of the cells that you get with any other method of making smears. This makes the very best cover glass smears I have seen. If you pick them out very critically and then force yourself to count how many cells are broken or disrupted, there are all kinds of variables that can be recognized. So if you count them, they'll turn out to be about 12 or 15 percent of all the leukocytes where the cell membranes are severely disrupted.

The remarkable thing there is absolutely no sign of sagging and the cells are uniformly flattened. The reason that they're so uniform is that when you put the blood on the slide the leukocytes and platelets stay precisely where they land and the red cells spin off. Now we have another interesting point concerning the matter of spinning speed. If one spins at speeds of about 5000 or 7000 rpm at the end, there will be areas where there is a blood clot and blood cells are perfectly beautiful in morphology. There is no displacement of intracellular contents at all. But, red cells come smashing up against them and against the blood platelets, and get deformed. They are often elliptical in shape and the central area may be displaced. If you just look at these you think they're beautiful for leukocytes but not good for red cells. You can get around this by spinning at 4000 rpm and putting a lid on them. Then you get a nice round red cell contour. But, my own feeling is that this effect is extremely important. This is a very easy way of studying red cell deformability. When you get an abnormal blood, you can see that the degree of deformability of the red cells is extremely variable from normal to abnormal specimens and among abnormal specimens. I think this might turn out to be very important.

DR. JACOBSON: Thank you, Dr. Ingram. Dr. MacEwen, did you have an additional question?

DR. MAC EWEN: Yes, I did. The second question I had, and I'm not sure I completely understood you this morning about the cell scan or the cell scan GLOPR. I got the impression that this was sort of a universal do-it-all and that if you put your blood smear on it you could obtain a red blood count, white blood count, a platelet count, a differential and perhaps even a reticulocyte count from the computer output. Is that correct?

DR. INGRAM: No, that is not quite correct. It is a system for classifying cells and getting quantitative measurements of certain morphological characteristics. It's really an image analyzer system that's concerned with the morphological characteristics of cell images.

DR. MAC EWEN: But it can differentiate between all of these cell forms?

DR. INGRAM: Yes.

DR. BEARD: I was just a little concerned about this spinning device you described as a possible way of making an aerosol of hepatitis virus.

DR. INGRAM: This is why it is now made with a cover. We actually made a cover for all of ours with a piece of safety glass over the top, but you can use it in a hood or with a dome over it, if you want to make deformability studies, and still be quite safe. We were aware of this hazard which is a very good point, as soon as we got the additional work done and published the paper on the method. The manufacturer then brought out the modified instrument with the cover on it. We could see this problem arising right away.