PROBLEMS IN AERIAL APPLICATION

Detection of Mild Poisoning by Organophosphorus Pesticides Using an Automated Method for Cholinesterase Activity

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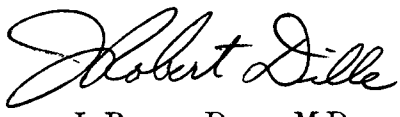

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Detection of Mild Poisoning by Organophosphorus Pesticides Using an Automated Method for Cholinesterase Activity

I. Introduction.

In 1950 Koelle[1] reported the use of thiolesters of choline in the histochemical detection of cholinesterase. Subsequently, many investigators have described various techniques for using these acetylcholine analogs as substrates for the quantitative measurement of cholinesterase in solution. Hydrolysis of acetylthiocholine has been followed by iodometric titration[2], decreased absorbance of the thiolester bond[3], and by reaction of the free SH group with nitroprusside[4]. By far the most sensitive and reliable method, however, was presented by Ellman *et al*[5] who measured cholinesterase by reaction of released thiol with 5,5′–dithiobis (2–nitrobenzoic acid) [DTNB]. A molar extinction coefficient of 13,600 was obtained at 412 mu.

A primary disadvantage of the manual techniques used for measuring the rate of hydrolysis of acetylthiocholine in the presence of blood and tissue homogenates is the high optical density due to the sample; for example, varying degrees of lipemia make it necessary to subtract a sample blank reading (no DTNB added) from the absorbance of each test mixture when plasma cholinesterase is measured. This disadvantage is minimized in the automated procedure reported in 1965 by Levine, Scheidt and Nelson[6]. In their technique a dialysis module is used so that only dialyzable substances, including unmasked thiol groups, can diffuse into a DTNB stream and thence into the light path of a colorimeter flow cell.

We have used the automated technique over the past year for the assay of cholinesterase activity in a variety of samples including blood and tissues of animals poisoned with organophosphorus and carbamate insecticides. We have found the method to be fast and reliable, but, as are most new methods, subject to certain pitfalls and errors which must be either corrected or kept in mind when the results are interpreted. In this report these problems will be considered.

The apparatus used in these experiments was exactly as described by Levine, Scheidt and Nelson[6] except that air was pumped into the DTNB side of the dialyzer through a .073 ID tube rather than the size used by the above authors. In all experiments the substrate concentration was 20 mM (as pumped).

II. Selection of the Sulfhydryl Standard.

Provided an adequate reaction time, almost any non-hygroscopic thiol compound of moderately high molecular weight may be used as a standard in the manual analysis of cholinesterase activity. However, the presence of a dialyzer membrane in the automated method imposes additional restrictions. For example, reduced glutathione, recommended as a standard by Levine, Scheidt and Nelson, produced an entirely different calibration curve when the substrate was being pumped into the system as compared to the slope produced when saline was substituted for acetylthiocholine solution.

The presence of this substrate produced no effect on the calibration slope for glutathione in the manual assay; therefore, it was concluded that acetylthiocholine in some way augmented the transport of glutathione across the dialysis membrane.

Other thiol standards were made by completely hydrolyzing acetylthiocholine perchlorate or iodide in weak alkali and then neutralizing with HCl. The data presented in Figure 1 indicate that identical slopes were obtained regardless of the presence of (unhydrolyzed) substrate. As thiocholine is the precise molecule to be measured in this method it seems most appropriate to use hydrolyzed substrate as a standard. This preparation may be diluted to appropriate concentrations and stored at –20°C for months without loss of sulfhydryl groups. A simple check for complete hydrolysis is to compare the solution

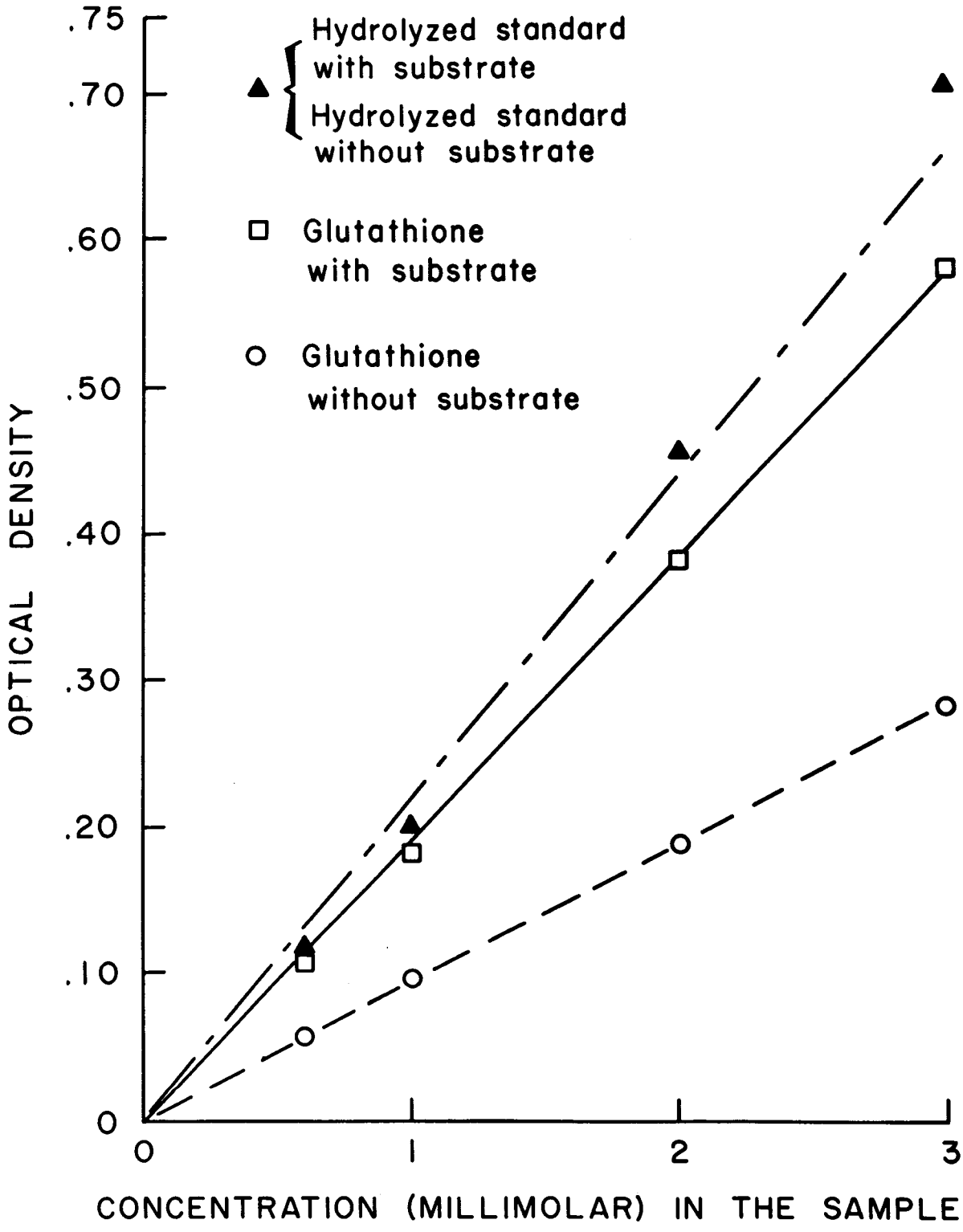

FIGURE 1. Evaluation of reduced glutathione and hydrolyzed substrate (acetylthiocholine) as standards. Lines marked □ and O indicate results with glutathione when substrate is pumped concurrently and when 0.9% NaCl is substituted. The line marked ▲ represents data obtained from hydrolyzed acetylthiocholine with and without the presence of unhydrolyzed substrate; in this case all points (2 for each condition) fell within the area of the triangle.

in a manual assay with appropriate concentrations of a primary standard such as reduced glutathione. Thiocholine iodide may now be purchased from the Pierce Chemical Company, Rockford, Illinois.

The apparent enhancement of the dialysis of glutathione by unhydrolyzed acetylthiocholine is interesting. Of course, we have not completely ruled out the possibility that glutathione causes hydrolysis of the substrate in the automated system, but the lack of influence by glutathione in the manual method makes this unlikely. The pH is quite stable in both methods, even in the presence of large concentrations of enzyme. In any case, the possibility of mutual interference during analytical dialysis should be considered when this module is employed. For example, in the analysis for total non-protein sulfhydryl present in a biological sample, one might be hard pressed to select a proper standard, for, not only might the dialysis of individual molecular species be influenced by other components of the sample, but, as dialysis is usually incomplete, some variability might be introduced through differences in rates of diffusion. Of course, this problem might not obtain in the analysis of individual compounds for which specific quantitative reactions are available, but recovery experiments would be necessary to rule out possible interference in dialysis even though this had already been checked in the manual procedure.

III. Calculation of the Enzyme Unit.

"One *unit* (U) of any enzyme is that amount which will catalyze the transformation of 1 micromole of the substrate per minute under defined conditions."[7] This unit, as defined by the Commission on Enzymes of the International Union of Biochemistry is more acceptable to us than the unit based on 3 minutes used by Garry and Routh in a manual method[8] and Levine, Scheidt and Nelson in the automated version of this method. A "3 minute" unit is even less meaningful in the automated procedure, for, although Garry and Routh employed a 3 minute incubation time ,the incubation time of approximately 4 minutes used in the automated method cannot be precisely known due to the complex analytical conditions; the enzyme and substrate, after mixing, pass through connecting tubing (not at 37°C) into an incubation coil (37°C) and then into the dialyzer module (37°C) where the products (and the substrate) are removed from the non-diffusable enzyme at an unknown rate. This removal of products may accelerate the enzyme reaction; on the other hand, the removal of some of the remaining substrate could slow the reaction rate. Thus, in order to make the data obtained from our automated analyses meaningful to those who do not possess this equipment, we established a conversion factor by analyzing several enzyme preparations by both the automated and a manual procedure. The enzyme solutions were made from a highly purified preparation of electric eel cholinesterase; these solutions did not absorb light at 420 mu, a decided advantage in the manual technique. The manual method used was essentially that of Gary and Routh except that we used phosdrin to stop the enzyme reaction. One example is given here to demonstrate the calibration procedure: A single dilution of electric eel cholinesterase was assayed by both methods. Hydrolyzed acetylthiocholine was used as the standard in each case. In the manual assay, after correction for chemical hydrolysis the enzyme concentration was calculated to be

$$\frac{590 \text{ umoles (SH)}}{\text{ml-min}}$$

or 590 U/ml. In the automated assay the same enzyme dilution produced a change in absorbance equal to a standard concentration of 2680 umoles (SH)/ml. Thus, the "true" or "effective" incubation time for the automated procedure may be calculated

$$\frac{2680 \text{ umoles}}{\text{ml}} \times \frac{\text{ml-min}}{590 \text{ umoles}} = 4.54 \text{ min.},$$

and this may be used to calculate units from experimental data (e.g.:

$$\frac{908 \text{ umoles}}{\text{ml}} \times \frac{1}{4.54 \text{ min}} = \frac{200\text{U}}{\text{ml}}).$$

Subsequent experiments have shown this factor to be constant over several months but changes in pumping rates and in the dialysis membrane would result in changes in the calibration factor and this must be watched for.

IV. Cholinesterase Inhibition in Poisoned Rats

Our main interest in the automated method was to employ it in the detection of organophosphorus poisoning in experimental animals. Thus. it was important to test this newer method against another, more established technique. Mr. Joe Rieger, Mr. Leonard Ryan and Dr. W. B. Stavinoha collaborated with us in a comparison

study using their procedure of measuring acetylcholinesterase by constant pH-titration. This method, although it possesses the advantage of low dilution of the sample, requires a longer analysis time; a skilled operator is able to perform about 40 analyses per day. Holtzman or Charles River albino rats weighing 150–170 gm were injected (i.p.) once daily with Disyston, 1 mg/kg. After 10 or 24 days of this treatment these rats and control rats that received daily injections of solvent, were beheaded at 24 hrs after the last injection. Brains were removed immediately and homogenized in cold Ringers solution; approximately ⅓ dilutions (precisely known) were analyzed by the titration method and approximately $\frac{1}{12}$ dilutions (precisely known) were assayed by the automated technique. Data from each poisoned rat were compared with cholinesterase (ChE) values for control animals killed at the same time. The cholinesterase activity for each rat is presented in Table I as percent inhibition, based on control values; this is necessary to compare data derived by the two methods because the substrate acetylcholine is hydrolyzed by rat brain cholinesterase approximately three

TABLE I. Inhibition of Brain ChE in Chronically Poisoned Rats.

Day†	*Rat*	*% Inhibition*	
		Constant pH Titration	*Autoanalyzer Method*
10	H*	74.7	79.7
		76.1	75.0
	C**	78.6	75.7
		81.3	76.9
24	H	78.4	78.5
		79.7	82.7
		77.8	74.7
	C	81.1	76.9
		79.6	81.7
		79.6	81.3

† Daily Injection of Disyston, 1 MG/KG For 10 or 24 Days
* Holtzman
** Charles River
Mean Difference = 0.38
t = 0.368
p = >0.5

times faster than the thiol analog. Statistical analysis of the data supported the hypothesis that identical evaluations of cholinesterase inhibition were obtained by both methods. Therefore, the automated technique would be useful in the assessment of organophosphorus poisoning in large numbers of samples.

V. Stability of Inhibition in Paraoxon-Poisoned Blood

Figure 2 demonstrates the usefulness of the automated procedure in generating families of curves when accurate timing is required.

In this experiment paraoxon was added to whole blood *via* microsyringe to the final concentrations indicated. Because sampling occured every 2 minutes the concentrations were prepared at 2 minute intervals to ensure equal incubation times. To evaluate the precision of the sampler cam we recorded the sampling times throughout the 240 minute experiment; the sampling interval never deviated by more than .04 minute throughout the experiment.

The data depicted in Figure 2 indicate that the cholinesterases in whole blood react slowly with paraoxon, equilibrium being reached at about 60 minutes; this rate shold be taken into account when blood cholinesterase activity is to be used as an index of poisoning.

There is some indication that inhibition was reversed after about 75 minutes when paraoxon concentration was 60 x 10^{-9} molar. This effect is consistent with the presence of a paraoxonase in blood as suggested by Augustinsson and Heimburger (9) and this is supported by data obtained in another experiment. Whole blood, plasma, and saline-washed erythrocytes were incubated for 100 minutes, long enough to reach equilbrium, with paraoxon at various concentrations. The results, presented in Figure 3, indicate that greater concentrations of paraoxon were required to inhibit the erythocyte enzyme when plasma was also present than when the washed cells were exposed to the inhibitor.

This effect of plasma could be due to the presence of a paraoxon-hydrolyzing enzyme or to paraoxon-reactive sites which are not associated with cholinesterase activity. In either case, if the plasma factor which modifies the inhibition of erythrocyte cholinesterase is variable between individuals, the cholinesterase activity of blood cannot be used as an indicator of blood paraoxon concentration. On the other hand, the extreme sensitivity of washed cells to paraoxon might be useful in measuring the concentration of this poison in protein-free solutions. We are presently studying the variability of the plasma fac-

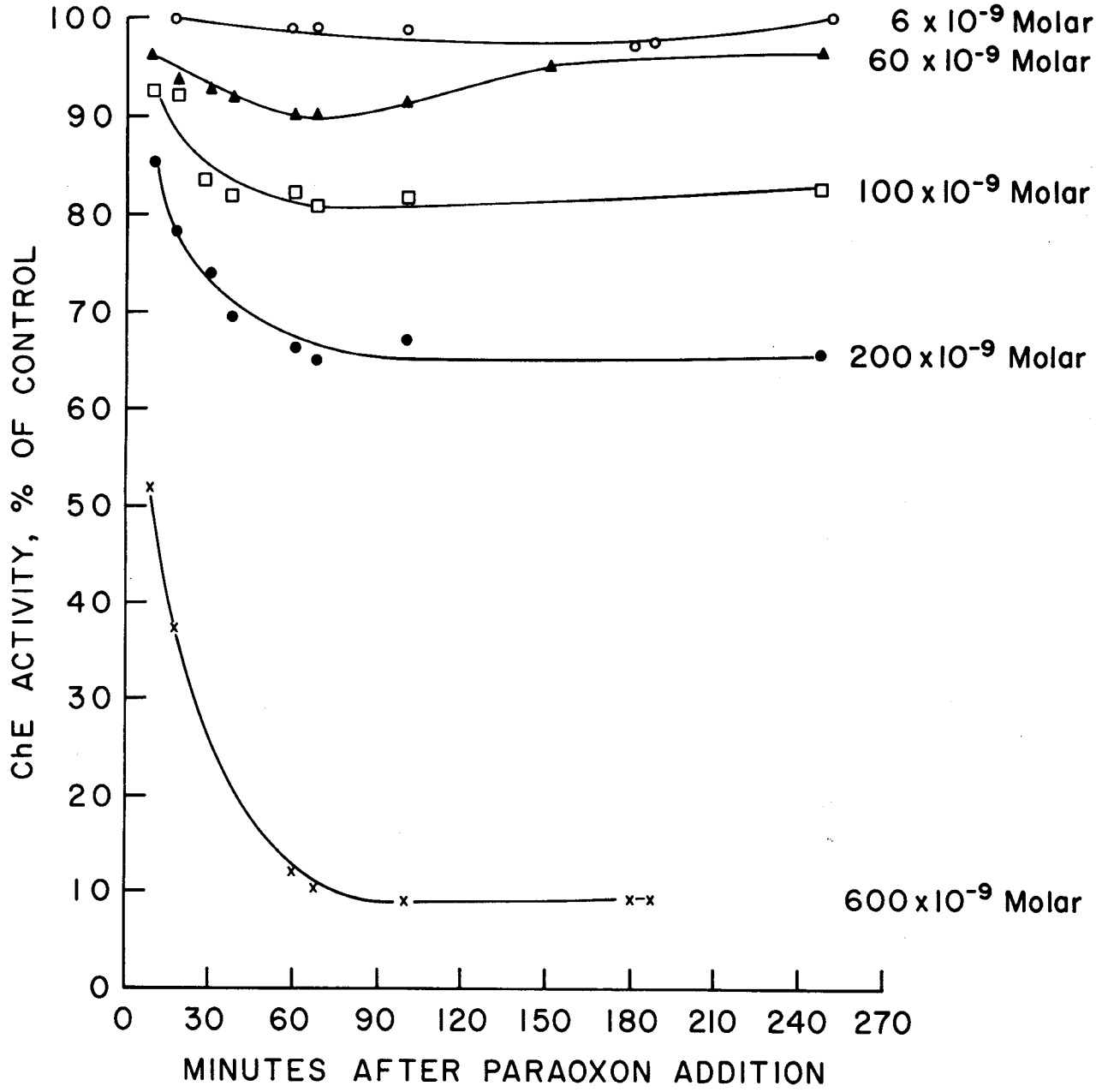

FIGURE 2. Inhibition of cholinesterase in whole human blood following the addition of paraoxon. The numbers at right indicate the final concentration in 99.8% blood Substrate conc. was 20 mM in reservoir.

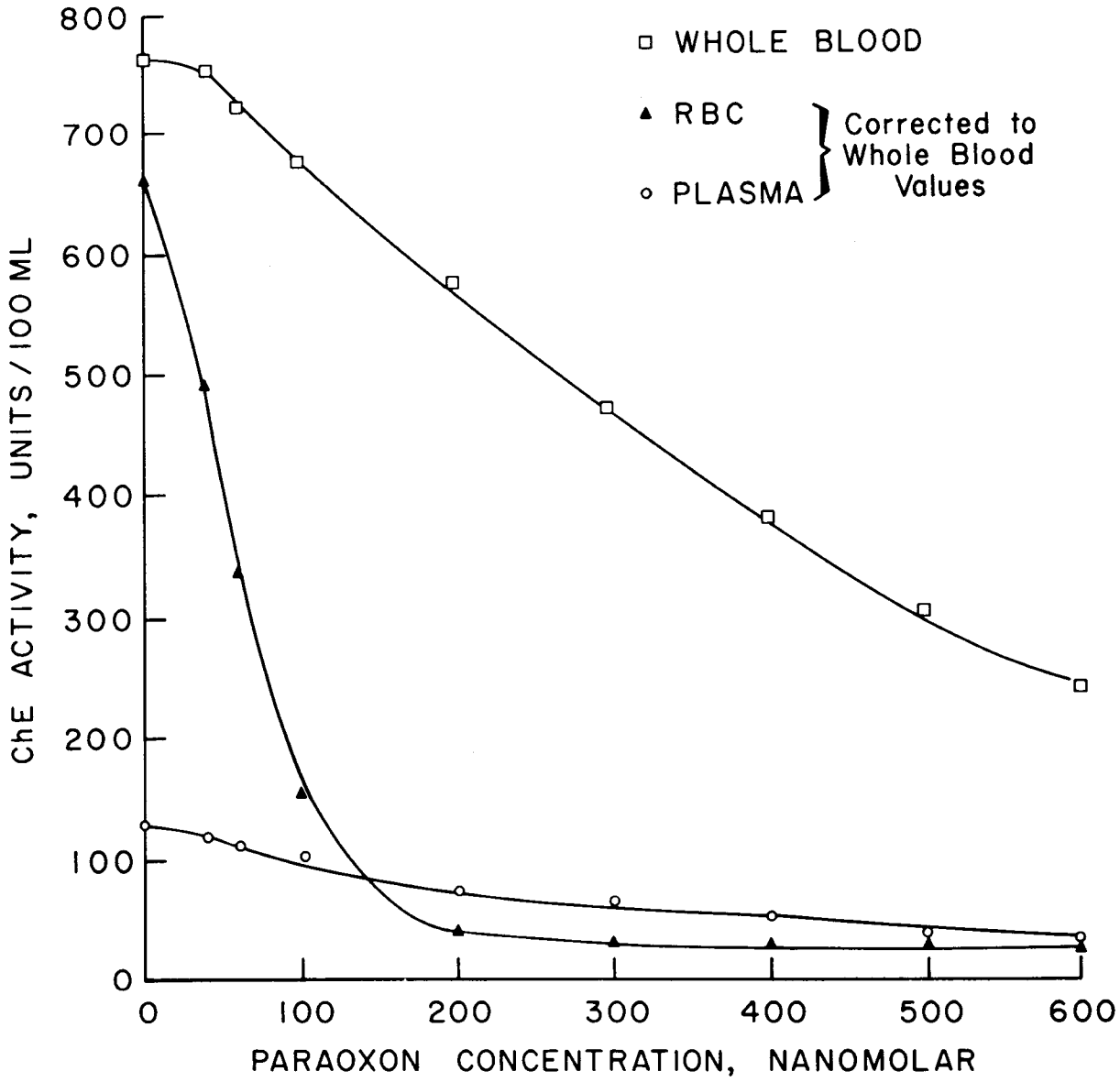

FIGURE 3. Protection by plasma of erythrocyte cholinesterase in the presence of paraoxon. Whole blood, plasma and washed erythrocytes from human blood incubated with paraoxon for 100 min. Erythrocytes washed and reconstituted to whole blood volumes with saline. Plasma ChE activities corrected to whole blood values using hematocrits. Note that plasma and RBC value may be added to obtain whole blood ChE activity in the unpoisoned samples, but not in paraoxon-exposed groups.

tor in man and experimental animals in order to evaluate its importance in the estimation of the magnitude of exposure to organophosphorus poisons.

VI. Stability of Inhibition by Carbamates

It is well known that the inhibition of blood cholinesterase by carbamate inhibitors may be partially reversed by dilution of the sample[10]. As the method of Levine, Scheidt and Nelson necessitates about a 1/15 dilution, we designed an experiment to evaluate the effects of dilution on the inhibition of whole blood by 1-naphthyl1-N-methyl carbamate (Sevin). Three concentrations each of Sevin and paraoxon were made up with whole blood; after 60 minutes the carbamate-poisoned samples were diluted with 1% Triton-X in TRIS buffer and the mixture was immediately taken up by the sampler. Each dilution was subsequently sampled every 8 minutes for 80 minutes. After 100 minutes of incubation the paraoxon-poisoned specimens were analyzed in the same way. Figure 4 presents the results in terms of percent of unpoisoned control which were analyzed concurrently.

Obviously, serious errors in the measurement of carbamate inhibition could occur if samples are not analyzed immediately upon dilution. This error could be minimized by sampling undiluted blood into a system which employs a much shorter incubation time, and we are presently investigating this possibility, but there seems to be no way to reduce the dilution which occurs in the apparatus when the sample is mixed with substrate, buffer, etc.; this amounts to an additional sample dilution of approximately 1/14. The constant pH titration method, however, imposed only a slight additional dilution (a total dilution of 1/4.5 for the entire assay) due to the addition of NaOH solution during titration of the released acetic acid. We attempted to evaluate the effects of the "built-in" dilution in the automated thiocholinesterase assay by comparing our results on eserine- and Sevin-inhibited samples to those obtained with the constant pH titrator; we are grateful to Mr. Leonard Ryan for data obtained by this latter method. This comparison is presented in Table II. One sample of heparinized human blood was divided into 9 parts and 3 concentrations each of paraoxon, Seven and eserine were made up. After allowing 30 minutes for the two carbamates and 100 minutes for paraoxon to equilibrate, the cells and plasma were separated and diluted in Ringer-Triton-X solution and analyzed immediately by both techniques. The data obtained from each poisoned sample were compared with those from non-poisoned blood assayed by the same procedures.

TABLE II. COMPARISON OF INHIBITION DATA OBTAINED BY THE AUTOANALYZER (AUTO) AND THE CONSTANT pH TITRATION (pH) TECHNIQUES

	ChE ACTIVITY— PERCENT OF CONTROL			
	RBC		*PLASMA*	
	AUTO	*pH*	*AUTO*	*pH*
PARAOXON				
150×10^{-9}M *	71	71	47	36
300×10^{-9}M *	42	42	18	14
450×10^{-9}M *	18	18	6	6
SEVIN				
0.5×10^{-5}M	66‡	60‡[1]	91†	78 †
1×10^{-5}M †	49	50	84	83
1×10^{-4}M *	22	13	30	29
ESERINE				
2.61×10^{-9}M †	95	90	95	88
1.3×10^{-8}M *	8	8	4	4
2.6×10^{-8}M *	5	3	2	2

* NO FURTHER DILUTION FOR AUTOANALYSIS OF ChE ACTIVITY
† ADDITIONAL ½ DILUTION FOR AUTOANALYSIS
‡ ADDITIONAL 1/3 DILUTION FOR AUTOANALYSIS

In Table II those columns marked with an asterisk present data which were obtained when no further dilution of the sample took place prior to automated analysis; other columns denote an additional ½ or 1/3 dilution over that used in the constant pH method. These data indicate that results obtained by the autoanalyzer technique are comparable to those obtained by the titrator method despite the fact that a much greater dilution of the sample is inherent in the former technique. It therefore seems likely that the automated dialysis method developed by Levine, Scheidt and Nelson would be useful in the detection of carbamate poisoning provided that samples are diluted just prior to analysis, or better, that a much shorter incubation time be employed so that prior sample dilution becomes unnecessary.

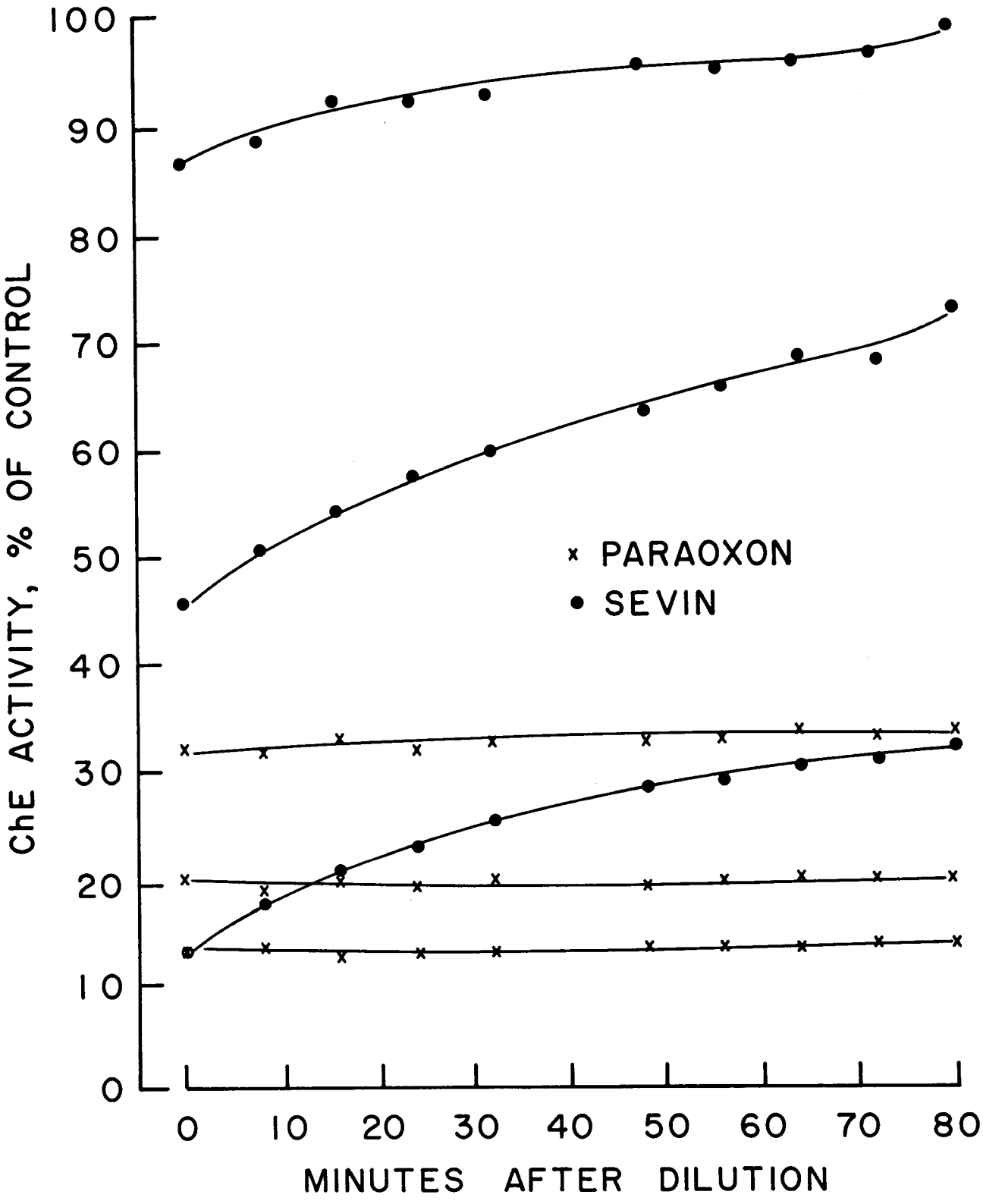

FIGURE 4. Reversibility of inhibition by Sevin after dilution of whole blood. Concentrations of Sevin (in undiluted sample) from top: 1, 10 and 100 x 10–6M. Concentrations of paraoxon: 3, 4 and 5 x 10–7M.

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ADAPTATION TO VESTIBULAR DISORIENTATION

V. Eye-Movement and Subjective Turning Responses to Two Durations of Angular Acceleration

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ADAPTATION TO VESTIBULAR DISORIENTATION

V. Eye-Movement and Subjective Turning Responses to Two Durations of Angular Acceleration

I. Introduction.

During prolonged angular acceleration, inertial torque deflects the cupula but the deflection is eventually balanced, primarily by the cupula's elastic restorative force. Assuming that cupula displacement from its position of static equilibrium controls the magnitude of vestibular reactions, a prolonged angular acceleration should, according to the "Torsion-Pendulum" theory[11], yield an increasing response for about 20–30 sec; this response level should be maintained without decline as long as the angular acceleration continues. Several authors [9,10,13,18,19] have reported that the subjective velocity rises and declines during prolonged angular acceleration, contrary to theoretical expectations. Guedry & Ceran[13] showed that the temporal period required for the subjective reaction to peak (and then decline) was about constant (27 sec) for angular accelerations ranging in magnitude from 0.5 to 2°/sec². Subsequent experiments (Guedry & Cunat, unpublished) showed this to be true for stimuli up to 4°/sec².

The present experiments seek to compare cat and man in regard to several characteristics of the nystagmic response elicited by two durations of a 4°/sec² angular acceleration.

II. Methodology.

A. Cats

Apparatus. The Huffman Rotation Device[6] located in a light-proof room, was used to produce acceleration programs. Animals were tested on the rotator in pairs with their heads at the center of rotation. One cat box was secured to runners on the turntable and the second box was secured to a framed tier arrangement above the first box.[7]

Restraint. Cats were restrained by the method of Henriksson, Fernandez, and Kohut.[16] Three or more days prior to testing, the animals were anesthetized and holes were drilled transversely through their canine teeth. At the same time, fur around the ocular orbits was shaved off and a guideline for positioning the head was drawn with washable ink from the canthus to the tragus on each side. For testing, each animal was wrapped in a towel and placed in a cat box. A strand of piano wire was inserted through the holes in the canine teeth. The wire was held securely and the head of the animal was positioned by means of an adjustable device on the front of the box.

Recording. For recording horizontal components of eye movements, needle electrodes were inserted by the outer canthi. Vertical components were obtained by means of surface electrodes taped above and below the left eye. The recorder was an Offner Type R Dynograph with 3-sec time constants used in amplification. Prior to testing, animals were placed in an optokinetic stimulator; a drum speed of 24°/sec was used to obtain data for calibration purposes.

B. Human Subjects

Apparatus. A Stille-Werner RS–3 rotating chair, situated in a light-proof room, provided the acceleratory stimuli for the human subjects.

Recording. A pair of surface electrodes, taped by the outer canthi of the eyes, detected horizontal eye movements, while a second pair was positioned above and below the left eye for the recording of vertical eye movement. An Offner Type T polygraph with a 3-sec time constant was used in amplifying and recording the eye movement signals. Eye calibrations were obtained prior to each test by means of a calibration chart located on one wall of the rotation room.

III. Procedure.

Each of eight cats and eight human subjects received 2 angular accelerations (for 8.4 and 36 sec) stimulating the lateral semicircular canals.

The same durations were used for the vertical canals. Stimuli were 4°/sec² accelerations and decelerations separated by 54 sec of constant velocity for cats and by 120 sec of constant velocity for humans. In cats, vertical canal stimulation was accomplished by placing each animal on its right side to locate its sagittal plane at the center and in the plane of rotation; human subjects leaned forward with the head turned to place the sagittal plane of the skull in the plane (and at the center) of rotation. A biteboard and head rest assisted in this positioning. Stimuli were presented in a counter-balanced order as indicated in Table 1.

Neither the cats nor the human subjects had been used in previous vestibular experiments. For the humans, this necessitated instruction regarding the signalling of subjective events without actual practice in making such judgments; they reported onset and cessation of apparent rotation by means of a signal key.

TABLE 1. Order of stimulus presentation. All trials comprised stimuli of 4°/sec². Duration of the stimulus was either 8.4 or 36 seconds. L and V refer, respectively, to lateral and vertical canal stimulation.

Human Subjects	*Cats*	*Rotation Direction*	*Trials* 1	2	3	4
Or & Wa	100 & 101	CW	8.4 L	36.0 L	8.4 V	36.0 V
Nu & Jo	102 & 103	CCW	36.0 L	8.4 L	36.0 V	8.4 V
Pe & Me	104 & 105	CW	8.4 V	36.0 V	8.4 L	36.0 L
Fr & Ma	106 & 107	CCW	36.0 V	8.4 V	36.0 L	8.4 L

IV. Results and Discussion.

The 8 animals received only 4 trials (2 lateral and 2 vertical canal stimulations) on the first day. Some examples of recorded nystagmus appear in Figure 1. The critical portion of the response for several purposes of this study began at the *end of each stimulus*. Thus, time measurements were made from the end of each stimulus (a) to the end of the primary response and (b) to the start of the secondary nystagmus. The number of beats of primary nystagmus which followed stimulus termination was also tabulated. These data appear in Table 2. In 47 of the 48 comparisons, the primary post-stimulus responses to the 8.4 sec stimulus exceeded those of the 36 sec stimulus.

TABLE 2. Measures of primary nystagmus following the termination of each rotatory stimulus for cats. Each response value is a mean of responses to an acceleration and a decleration stimulus. Stimuli were 4°/sec² for either 8.4 or 36 seconds.

	Time From End of Stimulus to End of Primary Nystagmus (Sec)				*Time From End of Stimulus to Start of Secondary Nystagmus (Sec)*				*Beats of Primary Nystagmus After End of Stimulus*			
	Lateral		*Vertical*		*Lateral*		*Vertical*		*Lateral*		*Vertical*	
Cat	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*
100	9.2	2.5	6.5	0.5	12.9	5.7	11.1	3.4	8.8	2.8	9.0	1.0
101	11.4	7.0	7.9	7.0	14.4	8.7	9.1	9.4	14.8	10.0	11.5	7.8
102	3.2	0.5	25.7	5.7	6.0	0.9	28.3	9.1	3.3	0.5	18.5	4.0
103	4.9	4.4	8.8	4.2	8.1	6.3	18.1	6.2	3.5	1.5	7.5	3.5
104	10.7	5.4	8.2	3.1	12.0	6.6	10.9	3.9	8.8	2.0	7.0	2.5
105	13.8	6.9	13.6	3.0	15.8	10.4	14.6	4.3	14.5	8.0	10.0	2.8
106	11.5	5.7	6.0	4.8	14.7	7.0	7.7	7.2	10.0	4.5	5.5	4.5
107	10.9	8.2	6.0	−1.5*	14.6	11.0	17.5	3.7	7.5	6.0	6.5	1.0
M=	9.5	5.1	10.3	3.4	12.3	7.1	14.7	5.9	8.9	4.4	9.4	3.4

* Nystagmus ended during stimulus.

Plots of the complete nystagmic responses to the 8.4 and the 36 sec stimuli appear in Figure 2. Slow-phase output was scored by summing the vertical displacements of beats from peak to base-line for successive 3-sec intervals. Greater output of primary nystagmus is evident for the lateral canals, as compared with the vertical canals, for both stimulus durations. For the 8.4 sec stimuli, primary nystagmus increased throughout the stimulus period for both the "horizontal" and "vertical" curves. For the 36 sec stimulus, there was a marked peaking in the response to stimulation of the vertical canals during the 15–18 sec interval, and a steady decline of that response throughout the remainder of the stimulus. For stimulation of the lateral canals during the 36 sec stimulus, peaking occurred in the 18–21 sec interval and was followed by a lesser decline than that noted for the vertical canals.

Secondary nystagmus was also plotted in Figure 2. With only one exception (the 8.4 sec stimulus to the vertical canals for cat No. 100), scorable secondary nystagmus was obtained from each cat for each stimulus condition. For both vertical and lateral canal responses, the 36 sec stimuli produced greater secondary responses than did the 8.4 sec stimuli. In addition, horizontal secondary nystagmus showed greater output than vertical secondary nystagmus by amounts proportional to differences in their respective primary reactions. Further, the mean peak of the secondary response occurred 21–24 sec after the end of the 8.4 and 36 sec stimuli for horizontal nystagmus, and 15–31 sec after the 8.4 and 36 sec stimuli for vertical nystagmus.

To pursue further the relationship of secondary to primary nystagmus and the effects of prolonged stimuli on those responses, six of the animals were given, one day later, a series of 15 trials stimulating the lateral canals with the 4°/sec² stimulus for 36 sec duration. Tracings for trials 1, 5, 10, and 15 were scored and the data plotted in Figure 3. With repeated stimulation, a marked depression of both the primary and secondary response curves occurred, peaking of the response was followed by a decline in nystagmic output during the remainder of the stimulus, and the peaks of both primary and secondary nystagmus shifted toward earlier occurrences.[5]

A. Human Subjects

Eight human subjects (4 males and 4 females) were given stimulations identical to those administered to the cats. Nystagmus data were also scored similarly and appear in Table 3. Some examples of nystagmus tracings are presented in Figure 4. In addition, duration of the sensation of turning was calculated from the *end of each stimulus* to the end point of the sensation (Table 4).

In 20 of the 48 comparisons (Table 3), human responses to the 36 sec stimulus exceeded responses to the 8.4 sec stimulus (9 of these cases were for the "number of beats" measure) and,

TABLE 3. Measures of primary nystagmus following the termination of each rotatory stimulus for human subjects. Each response value is a mean of responses to an acceleration and a deceleration stimulus. Stimuli were 4°/sec² for either 8.4 or 36 seconds.

	Time From End of Stimulus to End of Primary Nystagmus (Sec)				*Time From End of Stimulus to Start of Secondary Nystagmus (Sec)*				*Beats of Primary Nystagmus After End of Stimulus*			
	Lateral		*Vertical*		*Lateral*		*Vertical*		*Lateral*		*Vertical*	
Subject	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*	*8.4*	*36*
Nu	24.7	18.9	21.1	7.6	32.9	26.5	55.2	13.4	18.5	14.5	15.0	9.0
Or	29.8	34.7	16.1	26.5	32.0	36.6	9.8	9.0	92.0	61.5	25.5	23.0
Me	32.0	25.4	16.0	14.6	38.6	32.1	24.2	16.5	25.0	24.5	13.0	9.5
Wa	42.4	33.0	8.5	10.5	48.1	38.6	13.6	14.9	23.0	32.5	5.0	10.5
Pe	18.2	23.2	6.2	11.2	21.6	24.5	9.8	19.2	9.5	21.0	2.5	9.5
Fr	36.2	30.4	22.4	9.0	39.8	30.1	15.8	14.5	38.5	45.5	16.0	13.0
Jo	37.1	36.1	15.8	4.9	36.6	32.6	----	8.0	36.0	47.5	6.5	7.0
Ma	41.0	38.8	9.4	13.7	41.4	43.0	20.2	17.1	54.5	77.5	7.5	10.5
M=	32.7	30.1	14.4	12.3	36.4	33.0	21.2	14.1	37.1	40.6	11.4	11.5

although the mean group data for "duration of primary nystagmus" and for "time from end of stimulus to start of secondary nystagmus" were longer for the 8.4 sec stimulus, the differences were slight. Mean number of beats of primary nystagmus following stimulus termination actually favored the 36 sec over the 8.4 sec stimulus, but the differences were not significant. Thus, results obtained from the cats, in which the post-stimulus nystagmic responses to the 8.4 sec stimulus consistently exceeded those of the 36 sec stimulus, were not borne out in the data from human subjects.

TABLE 4. Time in seconds from end of each rotatory stimulus to end of subjective turning experience for uuman subjects. Each value is a mean for an acceleration and a deceleration stimulus. Stimuli were 4°/sec² for either 8.4 or 36 seconds.

	Lateral Canals		*Vertical Canals*	
Subject	8.4	36	8.4	36
Nu	----	----	----	----
Or	----	29.5	12.5	9.3
Me	34.5	12.4	44.0	9.2
Wa	27.8	14.9	10.4	23.7
Pe	11.0	6.7	7.7	2.0
Fr	23.1	14.3	11.5	28.6
Jo	20.4	12.4	19.9	−1.3*
Ma	7.7	3.1	7.4	−15.1*
M =	20.8	13.3	16.2	8.1

*Subjective turning experience ended during stimulus.

Human subjective data (Table 4), in 11 of 13 comparisons (3 comparisons were not obtained), showed that the 8.4 sec stimulus resulted in sensations of longer duration after termination of angular acceleration than did the 36 sec stimulus. For two subjects the sensation to vertical canal stimulation ended during the 36 sec stimulus.

Time plots of the nystagmus recorded during the two stimulus conditions appear in Figure 5. Responses from the lateral canals were of greater magnitude than those from the vertical canals. No clear peaking or decline in output during the stimulus appeared for either set of canals. Secondary nystagmus was plotted in the same figure. However, of the 8 subjects, 6 gave no scorable secondary responses to the 8.4 sec lateral canal stimulus, 5 gave none to the same stimulus applied to the vertical canals, 3 gave none to the 36 sec vertical canal stimulus, and one gave no scorable secondary nystagmus to the 36 sec lateral canal stimulus. Thus, in two general respects, human data differed from cat data: 1) humans did not show a rise and decline during prolonged (36 sec) stimulation whereas the cats did; 2) little or no secondary nystagmic reactions to the 8.4 sec stimuli were demonstrated by humans whereas cats consistently gave such responses.

Arousal. The importance of arousal on nystagmic responses was noted earlier and has been examined in considerable detail elsewhere.[3] To assure reliability of the present data with regard to this factor, a second group of 10 cats was treated with d-amphetamine in accordance with procedures described by Crampton and Brown.[8] Each animal received 4 trials with each trial comprising an acceleration stimulus of 4°/sec² for 36 sec, 2 min of constant velocity, and a subthreshold deceleration (0.15°/sec²). The first two trials were always stimulation of the lateral canals; the remaining two trials involved vertical canal stimulation. Trials were alternately CW and CCW. Half the animals began with CW rotation; the remaining 5 began with CCW rotation. Data from CW and CCW accelerations were similar for the horizontal and the vertical nystagmus curves and, therefore, were averaged. The mean response curves for the 10 animals appear in Figure 6 and demonstrate the same type of decline during stimulation and almost identical transition points from primary to secondary nystagmus as those presented by the undrugged cats. Vertical nystagmus again shows a more pronounced decline during stimulation than does horizontal nystagmus. Some supportive neural data for this decline of response during stimulation of the cat has been presented by Cappel[2] who showed plots of single neural unit activity during a prolonged angular acceleration and reported a rise and decline of firing during the stimulus period.

A reliability check of the findings for humans was accomplished by exposing 4 males and 4 females, all previously untested, to stimulus conditions (4°/sec² for 36 sec) identical to those administered to the other human subjects. Half of the males and females received CW stimulation, the remaining half received CCW rotation. Four trials were administered, two each for the lateral and vertical canals. The first two trials always employed mental arithmetic (MA) as

an arousal task[3], while in the last two trials, the Key Press (KP) technique of estimating subjective velocity was used to maintain alertness. With the KP technique, the subjects attempted to signal successive angular displacements of 90°. Table 5 contains an outline of the test procedures for this reliability check. Nystagmus data for acceleration and deceleration and for the two tasks showed no evidence of a fall-off in response during stimulation, and the primary-to-secondary nystagmus transition points were almost identical to those obtained under the first set of conditions. The data were thus combined and curves depicting horizontal and vertical nystagmus were plotted in Figure 7. As in the first group of human subjects, no clear evidence for a decline of response during the stimulus is evident. However, the vertical nystagmus time-plot does show considerable irregularity as compared with horizontal nystagmus data. Thus, the differences between man and cat that were obtained from the first groups of subjects were confirmed with different subjects and under conditions in which the arousal variable was manipulated.

TABLE 5. Order of stimulus presentation for human subjects used in the reliability check. Arousal of subjects was controlled by Mental Arithmetic and Key Press tasks. All trials comprised accelerations and decelerations of 4°/sec² for 36 sec separated by 2 min of constant velocity.

Subjects		*Rotation*	*Mental Arithmetic*		*Key Press*	
Male	*Female*	*Direction*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*
A	W	CW	Lateral	Vertical	Vertical	Lateral
B	X	CCW	Lateral	Vertical	Vertical	Lateral
C	Y	CW	Vertical	Lateral	Lateral	Vertical
D	Z	CCW	Vertical	Lateral	Lateral	Vertical

It has been reported that vestibular nystagmus rises and declines, like the subjective reaction, during constant angular acceleration.[1,17,20,21] However, Guedry & Lauver[15] demonstrated that the nystagmic reaction in man did not decline during prolonged constant angular acceleration if the subjects were required to signal estimates of angular displacement. Occasionally, however, a subject would yield a rise and decline in the nystagmus response similar to the subjective responses in earlier experiments (see Figure 4 in Guedry & Lauver[15]). To check the possibility that arousal accounted for the variety of findings, Collins & Guedry[4] required subjects, during prolonged angular accelerations, to perform mental tasks, which would maintain mental activity independent of the subjective perception of rotation. Subjects were required to make arithmetic computations throughout the vestibular stimulation and post-stimulation periods. Results showed again that nystagmus first increased and then remained constant during constant angular acceleration. Following the termination of stimulation, nystagmus decayed about as expected from the "torsion pendulum" theory[11], although rates of decay were not calculated. The same subjects, when allowed to relax, occasionally showed a rise and decline of nystagmus during constant stimulation, and a rapid decay of response on termination of the stimulus. The present experiments confirm the fact that nystagmus does not decline during prolonged angular acceleration in alert human subjects, although such declines appear to occur in cats.

In cats, time to *onset* of secondary nystagmus was inversely related to duration of the stimulus. The earlier *onset* of the secondary reaction cannot be attributed to loss of arousal. This result strongly suggests that the decline in primary nystagmus during and after the longer stimuli resulted from a counteracting process which reduced the intensity and duration of the primary nystagmus and then became evident as an early secondary nystagmus. Data from humans have shown that as the duration of a constant angular acceleration is increased beyond certain time limits, the duration of the subjective after-responses becomes shorter and shorter.[12,14] Nystagmic reactions in cats showed similar effects and, in these respects, more closely resembled the subjective reactions of man than they did the nystagmus of man.

V. Summary.

Recordings of ocular nystagmus were obtained from a group of cats and a group of human subjects to 4°/sec² angular accelerations of 8.4 sec and of 36 sec duration. Lateral canals and vertical canals were stimulated on separate trials. Results showed that the output of both primary and secondary nystagmus was greater for lateral canals. In cats, both lateral- and vertical-canal responses to the 36 sec stimuli peaked after 15–21 sec of angular acceleration and this was followed by a steady decline. Declines were not apparent in nystagmus of human subjects. A further test of these findings was conducted by manipulating arousal variables; human subjects were given special tasks and cats received d-amphetamine. Essentially the same results were obtained as described above. Other differences between the two groups were noted. Cats consistently demonstrated secondary nystagmus whereas humans did not. After termination of acceleration, primary nystagmus from cats lasted longer and exhibited a greater number of eye movements following the 8.4 sec stimulus than following the 36 sec stimulus; this consistency was not evident in humans. However, for humans the sensation of motion following termination of acceleration was of longer duration for the 8.4 sec stimulus than for the 36 sec stimulus. In this regard, nystagmus from cats resembled the subjective reactions of man more than they did the nystagmus of man.

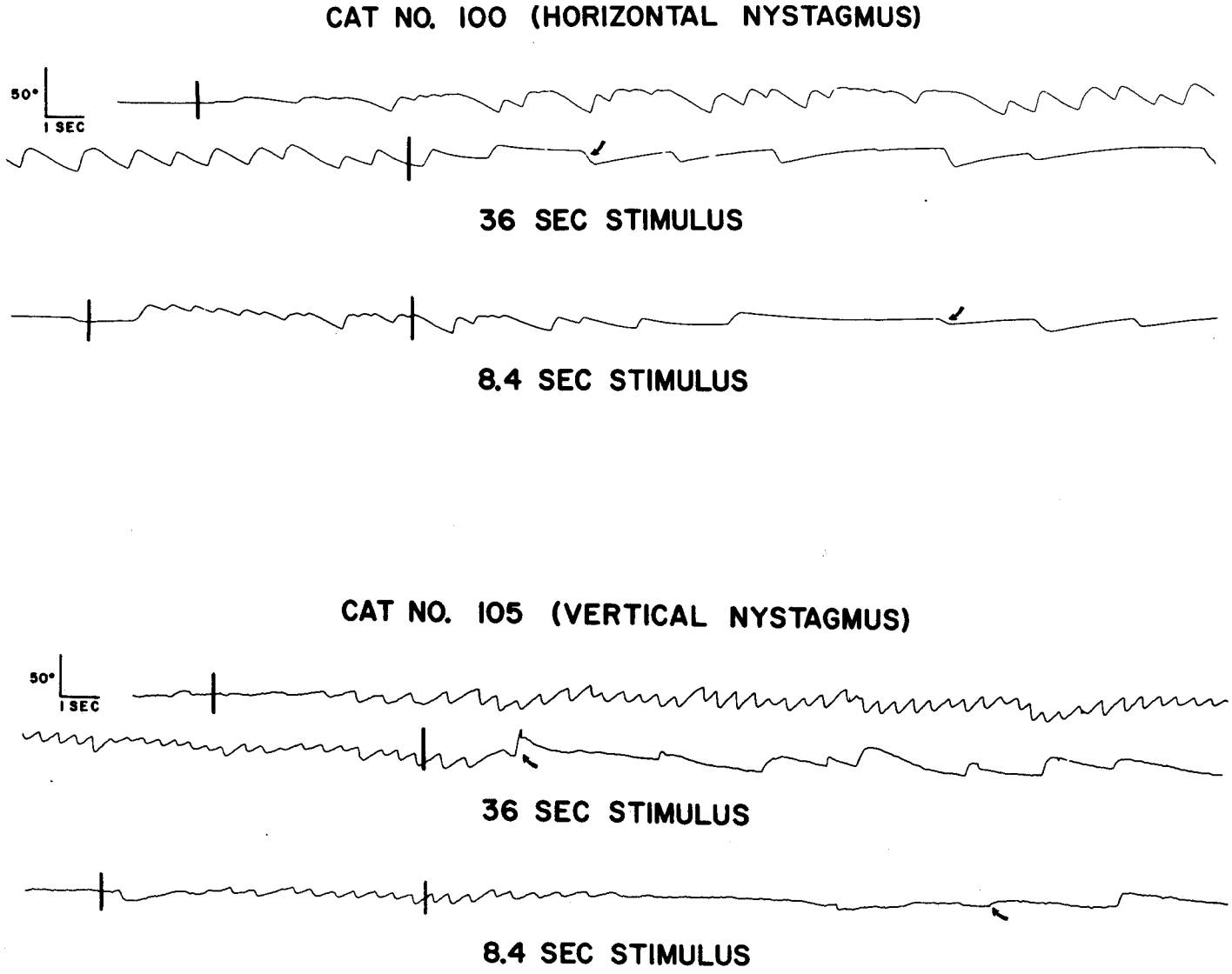

FIGURE 1. Nystagmus recorded from two cats for two durations of a 4°/sec² angular acceleration. Responses to stimulation of the lateral canals (Cat No. 100) and the vertical canals (Cat No. 105) are presented. Vertical bars through the tracings demarcate the stimulus periods; arrows indicate the start of secondary nystagmus. Note the longer poststimulus primary nystagmus and the later onset of secondary nystagmus for the shorter duration of acceleration.

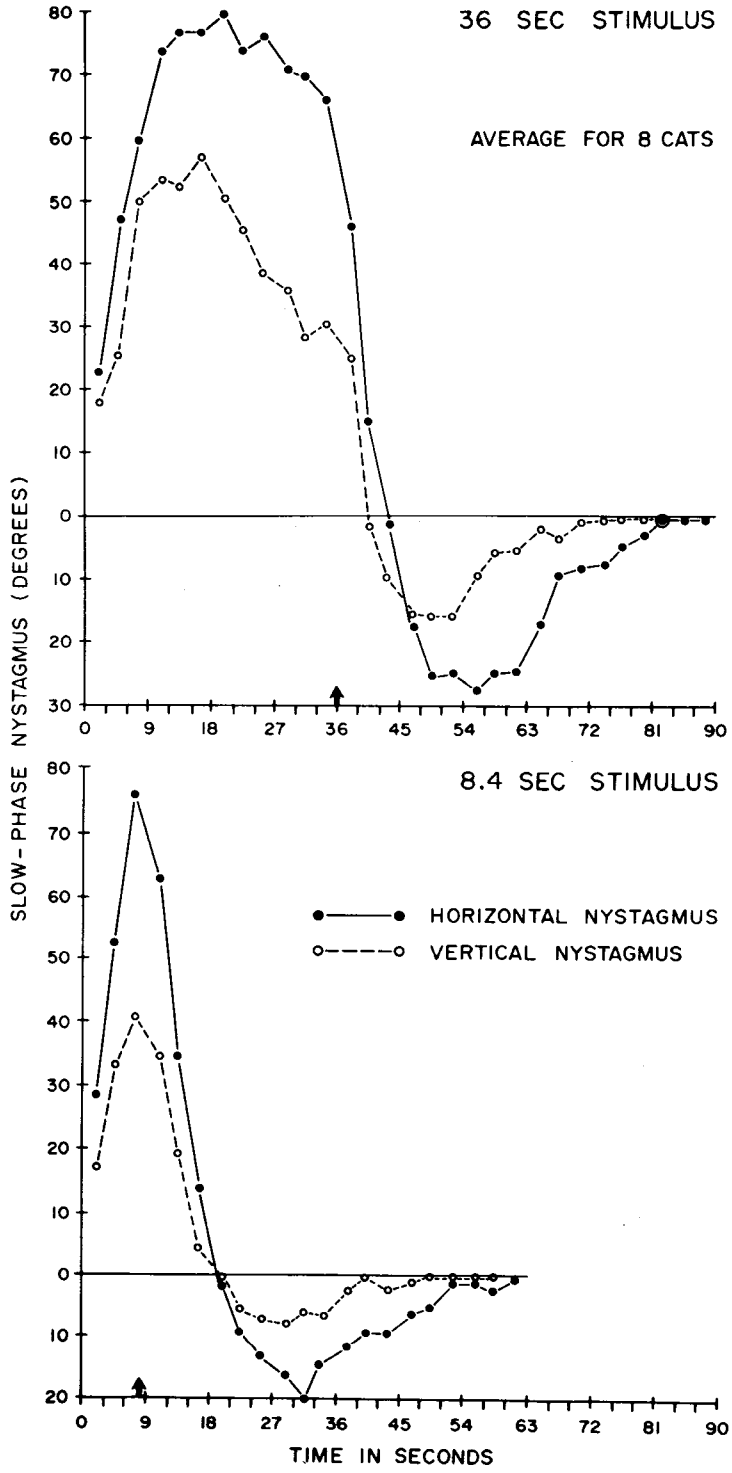

FIGURE 2. Time-course plots of slow-phase eye displacement in 3-sec intervals for 8 cats. Arrows indicate termination of acceleration. Points above the zero line represent primary nystagmus; points below the zeroline represent secondary nystagmus. In the upper graph, a clear peaking and decline of nystagmus during the acceleration is evident for responses to both lateral- and vertical-canal stimulation. In the lower graph (8.4 sec stimulus), 3-sec intervals were marked off from the *end* of the stimulus (thus the first "3-sec" interval represents response during only 2.4 sec of stimulus). Total output of both primary and secondary nystagmus is consistently greater for the lateral canals.

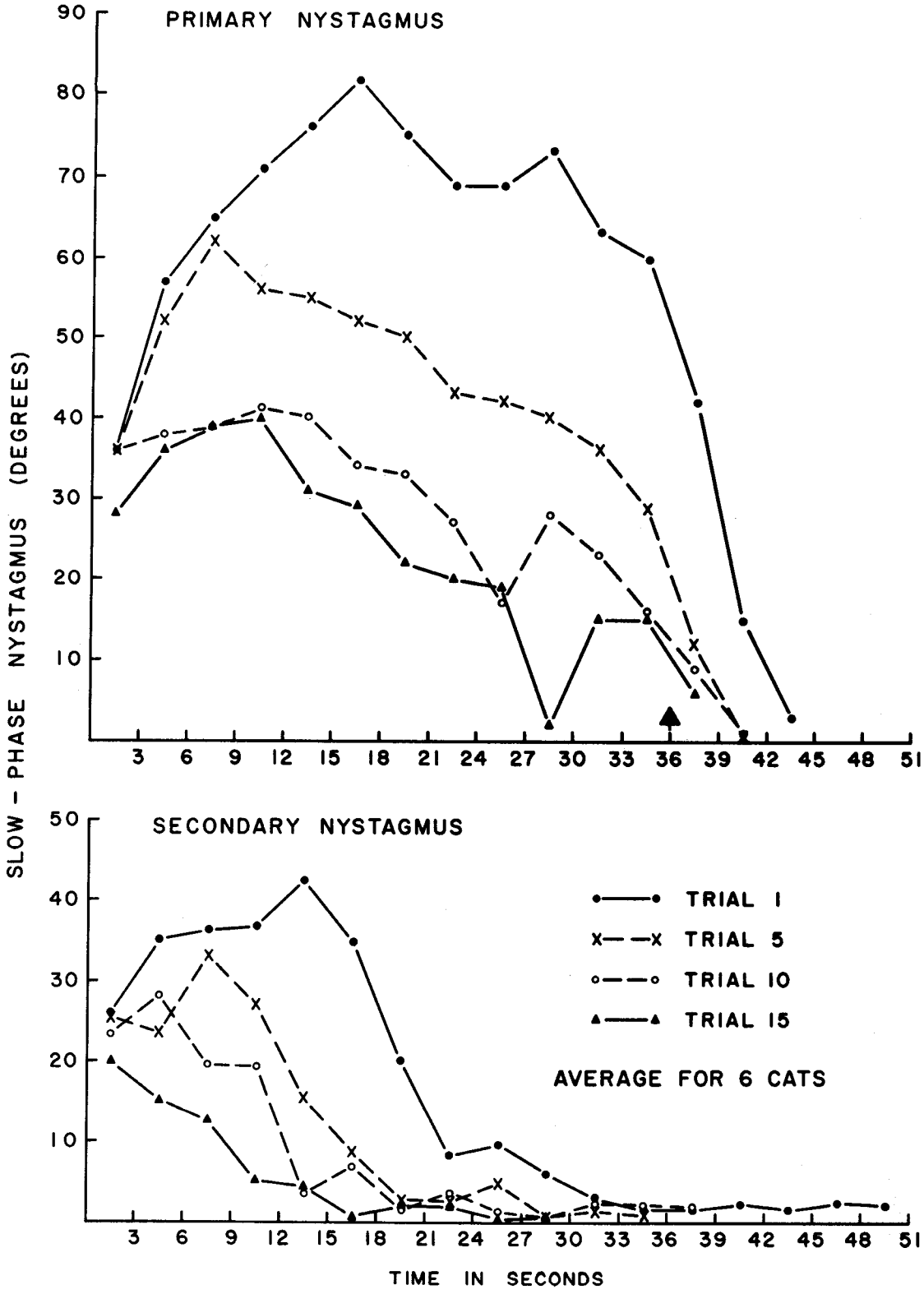

FIGURE 3. Time-course plots of slow-phase eye displacement for trials 1, 5, 10, and 15 of the habituation series. With repeated trials there occurs a depression of output with early peaking followed by rapid declines during the accelerations. Effects are similar for both primary and secondary nystagmus.

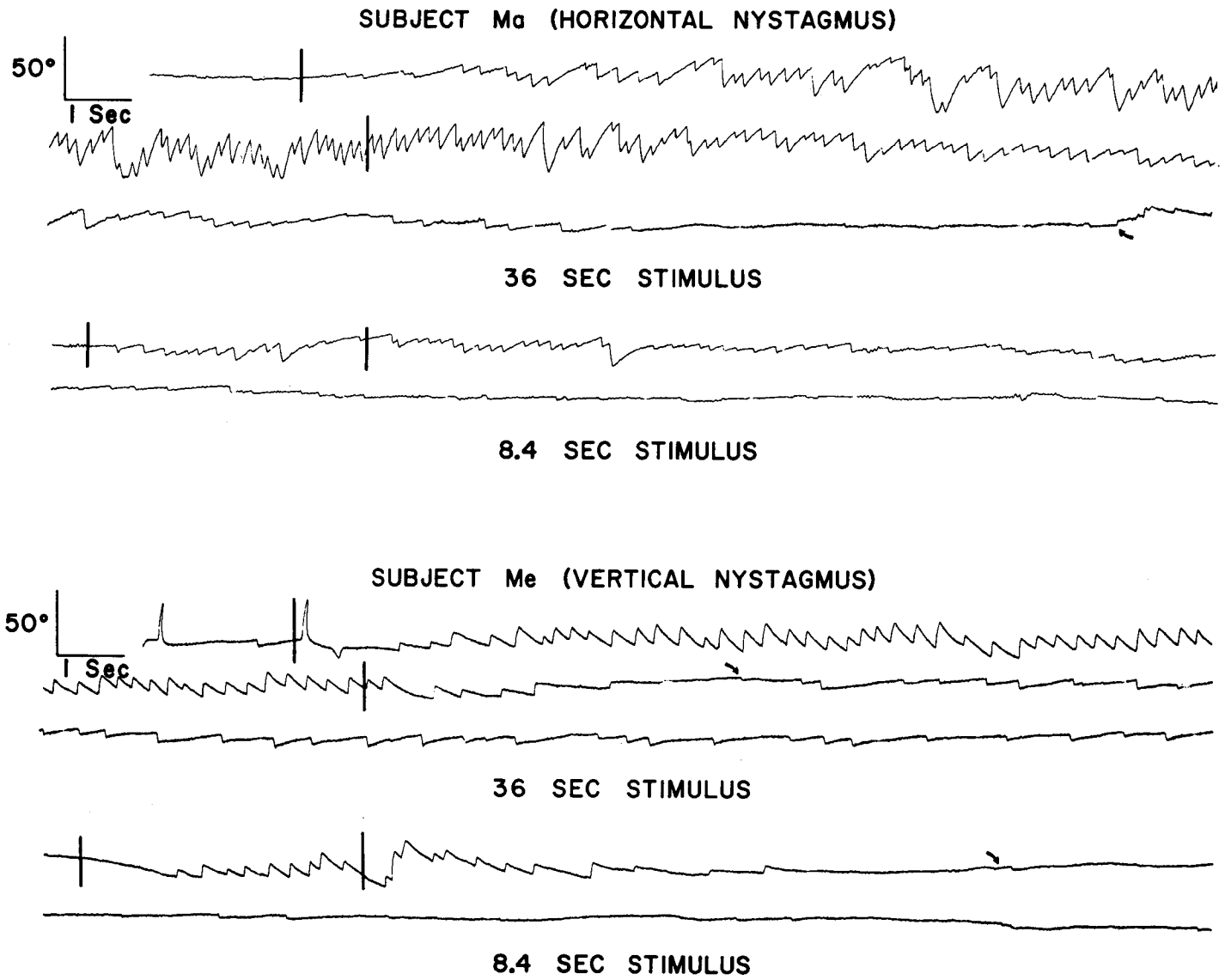

FIGURE 4. Nystagmus recorded from two human subjects for two durations of a 4°/sec² angular acceleration. Responses to stimulation of the lateral canals (Subject Ma) and of the vertical canals (Subject Me) are presented. Markings are the same as in Figure 1. The clearly longer responses of the lateral canals to the shorter stimulus duration which were obtained from cats are not so evident here (compare Subject Ma data with Figure 1). Subject Me (vertical nystagmus) was atypical of human subjects in demonstrating clear and consistent secondary nystagmus with tracings closely resembling those obtained from cats.

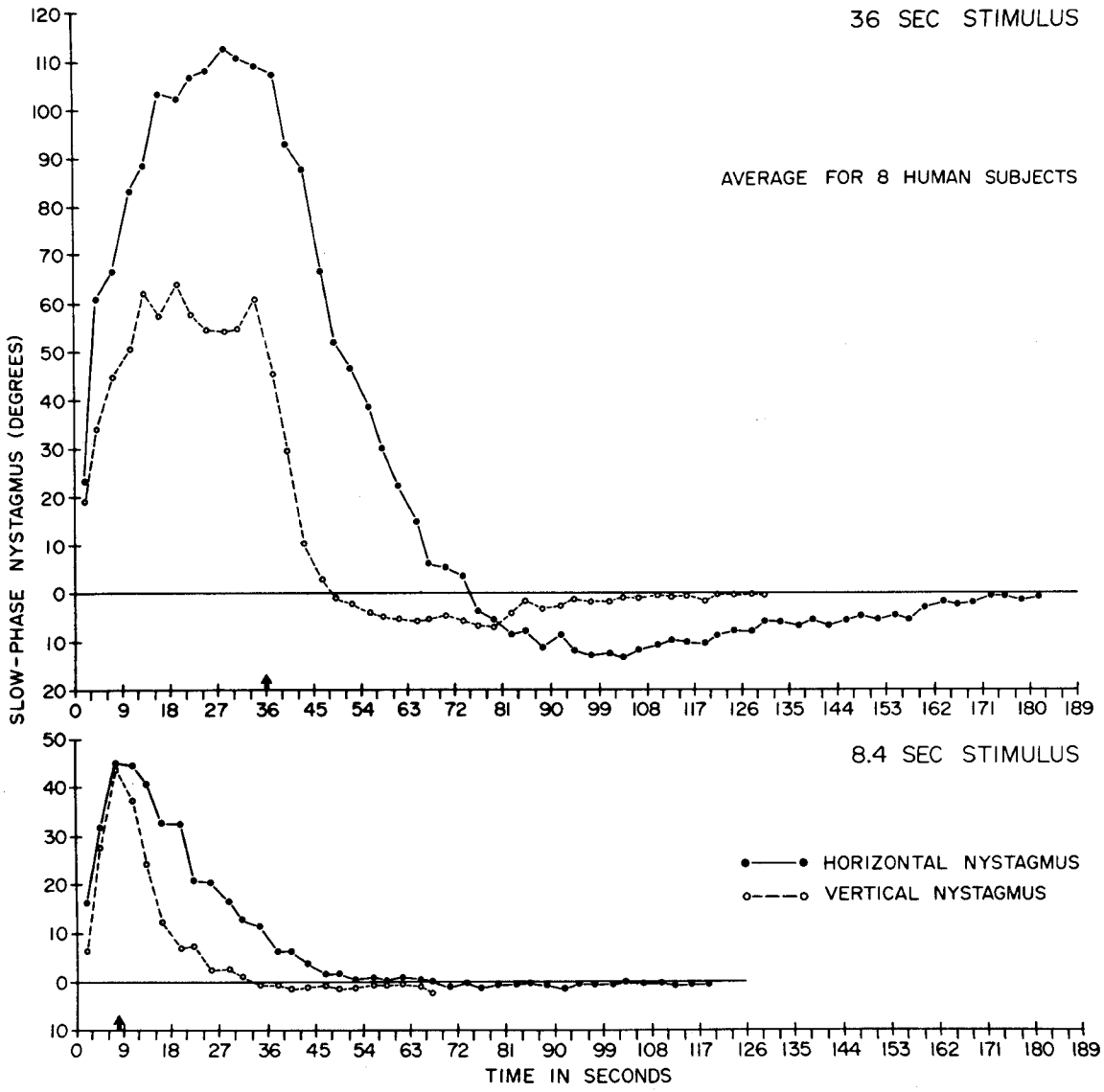

FIGURE 5. Time-course plots of slow-phase eye displacement in 3-sec intervals for 8 human subjects. Markings are the same as in Figure 2. Total output of both primary and secondary nystagmus is greater for the lateral canals. In the upper graph, no clear peaking and subsequent decline of nystagmus during the acceleration is evident, although a large dip appears in the data for the vertical canals (compare with Figure 7).

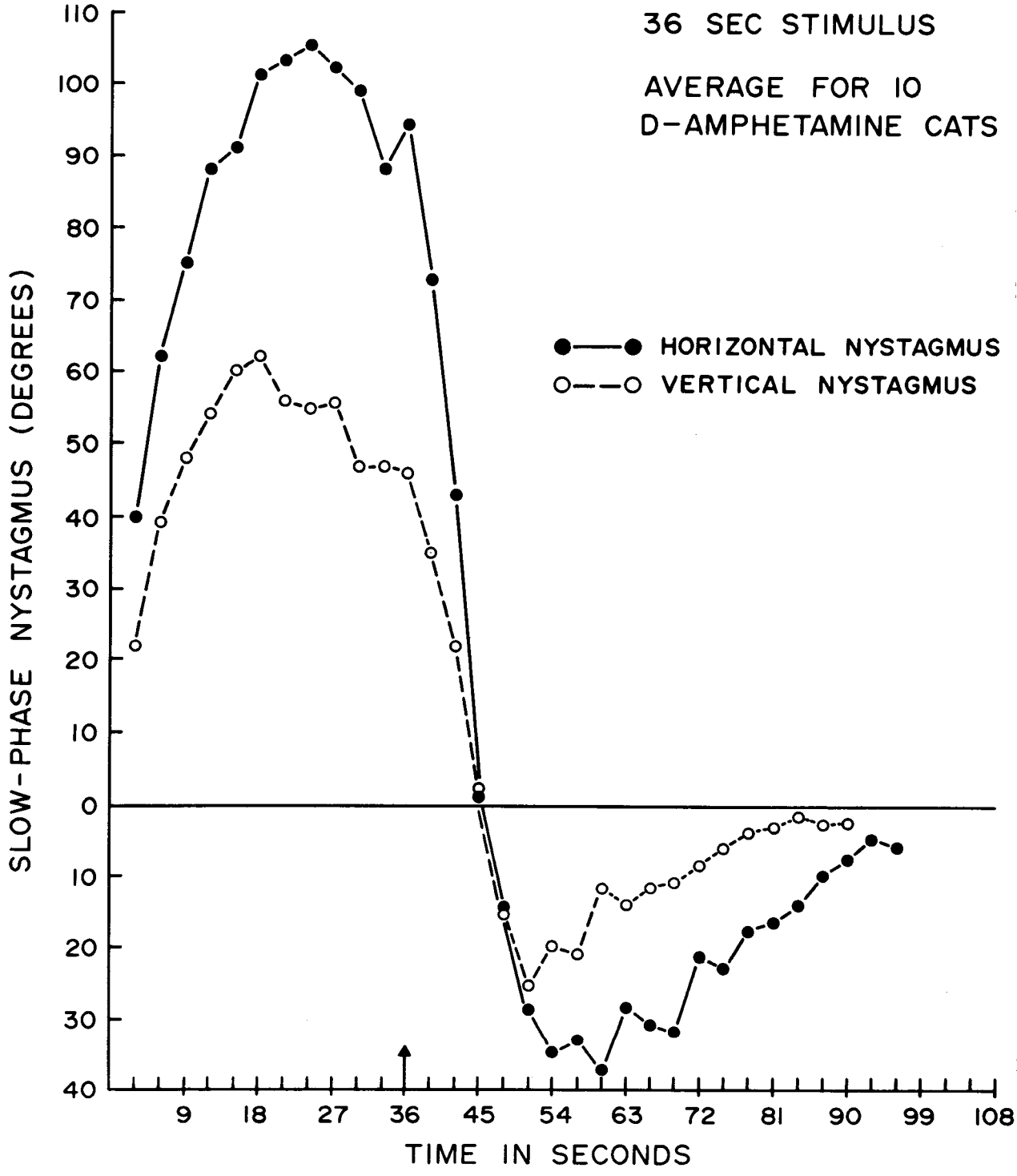

FIGURE 6. Time-course plots of primary and secondary nystagmus in 3-sec intervals for cats treated with d-amphetamine. Similar to results obtained from undrugged cats, a peaking and decline of the response during the 36 sec stimulus (4°/sec²) is evident for both lateral- and vertical-canal stimulation (compare with Figure 2).

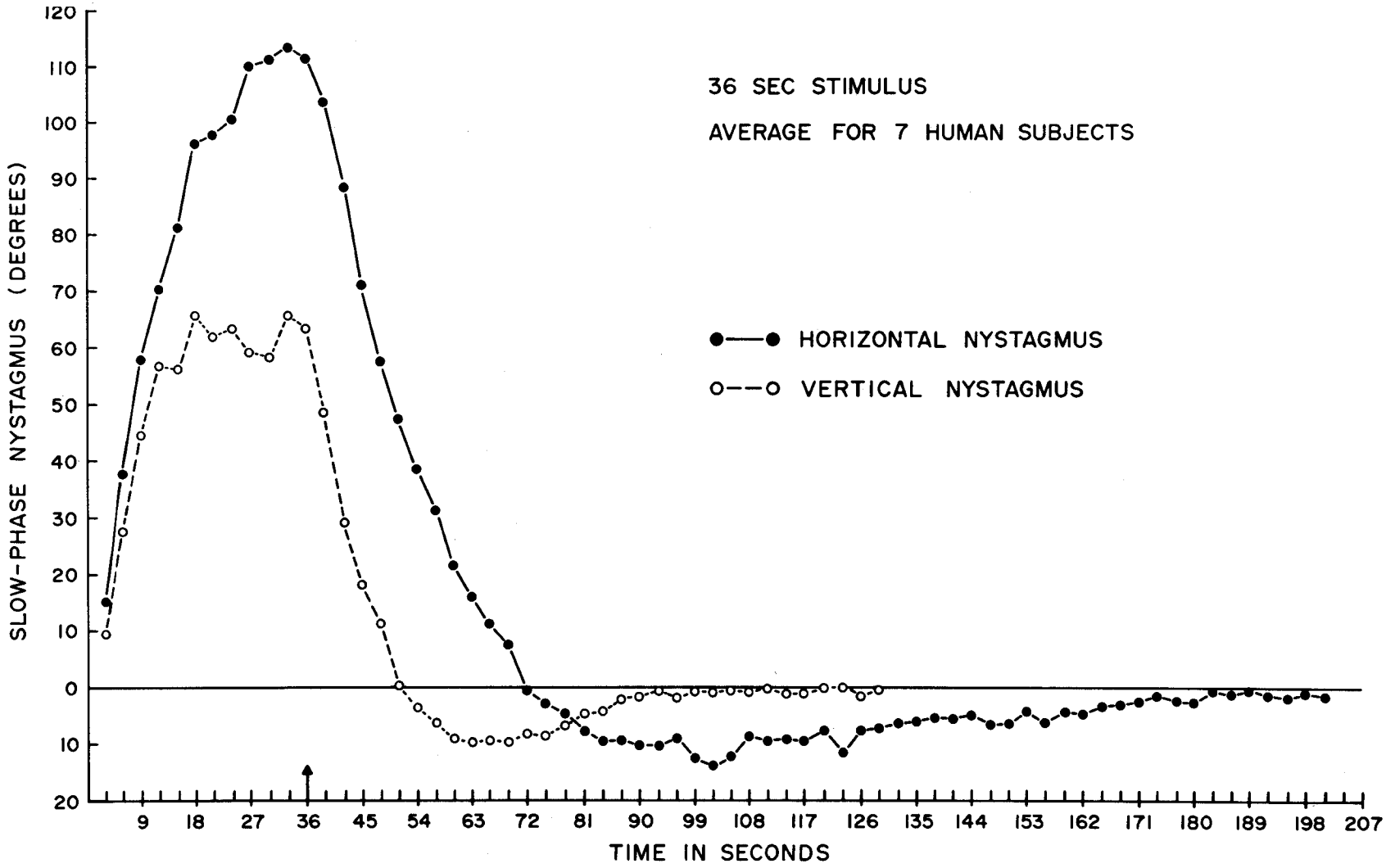

FIGURE 7. Time-course plots of primary nystagmus in 3-sec intervals for a second group of humans who were given instructions influencing arousal during the tests. Stimuli were 4°/sec² angular accelerations for 36 sec. Each point is an average of two task-trials (metal arithmetic and key pressing) for each of the 8 subjects. Similar to results obtained under other arousal conditions (compare with Figure 5), no peaking and subsequent decline of nystagmus during accelerations is evident. One female subject was excluded from the average curves due to the presence of a spontaneous nystagmus which particularly affected scoring of secondary responses.

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ADAPTATION TO VESTIBULAR DISORIENTATION

VI. Eye-Movement and Subjective Turning Responses to Varied Durations of Angular Acceleration

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ADAPTATION TO VESTIBULAR DISORIENTATION

VI. Eye-Movement and Subjective Turning Responses to Varied Durations of Angular Acceleration

I. Introduction

Ocular nystagmic responses elicited by angular acceleration have been found to be different in cat and in man.[6] With prolonged stimuli (36 sec), a rise and decline of nystagmus during stimulation was obtained from cats for both lateral- and vertical-canal stimulation. Human subjects did not exhibit a comparable decline during prolonged stimulation. Nystagmic reactions of the cat thus resembled the subjective (rather than the nystagmic) reactions of human subjects.[9,10,12,19,20]

At least part of the difference noted above in regard to primary nystagmic reactions appears related to the prominent secondary nystagmus which characterizes responses of the cat to angular accelerations. Secondary nystagmus is opposite in direction to the primary response, and it may reflect a process which develops during, and opposes continuation of, the primary reaction.

The present investigation represents an extension of the earlier study.[6] A range of stimulus durations was used to clarify relations between stimulus duration, the rate of decline of primary nystagmus, and the intensity of secondary nystagmus, and to compare further vestibular processes in cat and man.

II. Methodology

A. Cats

Apparatus. Rotational stimulation was provided in a light-proof room with the Huffman Rotation Device.[7] Angular accelerations and decelerations were 4°/sec² separated by 54 sec of constant velocity. Animals were tested in pairs (with their heads at the center of rotation) by means of a set of tiers.[8]

Restraint. Restraint was effected by the method of Henriksson, Fernandez, and Kohut[15] and in the manner described elsewhere.[6]

Recording. An Offner Type R Dynograph recorded horizontal components of eye movements from needle electrodes inserted by the outer canthi. A 3-sec time constant was used in amplification.

B. Human Subjects

Apparatus. A Stille-Werner RS–3 rotator was programmed to provide accelerations and decelerations of 4°/sec² separated by 2 min of constant velocity.

Recording. An Offner Type T polygraph (time constant: 3 sec) recorded horizontal components of eye movements from surface electrodes taped by the outer canthi.

III. Procedure

Each of 12 cats and 12 human subjects received 6 rotatory trials comprising 4°/sec² accelerations and decelerations for 1.2, 3, 9, 15, 21, and 30 sec. Rotation was always counterclockwise and the order of presentation of the stimulus durations was counterbalanced among pairs of subjects as indicated in Table 1.

TABLE 1. Order of presentation of stimulus durations (in seconds). Durations varied from 1.2 to 30 sec. All angular accelerations were 4°/sec² and rotation was always counterclockwise. Only the lateral canals were stimulated.

Human		*Trials*					
Subjects	*Cats*	*1*	*2*	*3*	*4*	*5*	*6*
Pz & Ch	108 & 109	1.2	3.0	9.0	15.0	21.0	30.0
Dy & Do	110 & 111	3.0	9.0	15.0	21.0	30.0	1.2
Sa & Br	112 & 113	9.0	15.0	21.0	30.0	1.2	3.0
Dt & Da	114 & 115	15.0	21.0	30.0	1.2	3.0	9.0
Te & Ro	116 & 117	21.0	30.0	1.2	3.0	9.0	15.0
Ve & Pa	118 & 119	30.0	1.2	3.0	9.0	15.0	21.0

None of the subjects had been used in previous vestibular experiments. Human subjects (6 men and 6 women) were instructed to signal onset and cessation of their rotatory experiences by means of a signal key.

Scoring. Slow-phase displacement of the eyes was scored by measuring the peak to base-line distance for each beat of nystagmus and summing these values for 3-sec intervals. Time measurements were also made from the end of each stimulus (a) to the end of the primary response and (b) to the start of the secondary nystagmus. The number of beats of primary nystagmus which followed stimulus termination was tabulated.

IV. Results and Discussion

A. Cats

Measures of both response time and the number of beats of primary nystagmus following stimulus termination appear in Table 2. Post-stimulus responses of greatest duration and magnitude occurred when stimulus durations were between 3 and 15 sec with the maximum post-stimulus primary response occurring, in general, with stimuli of 9 sec duration.

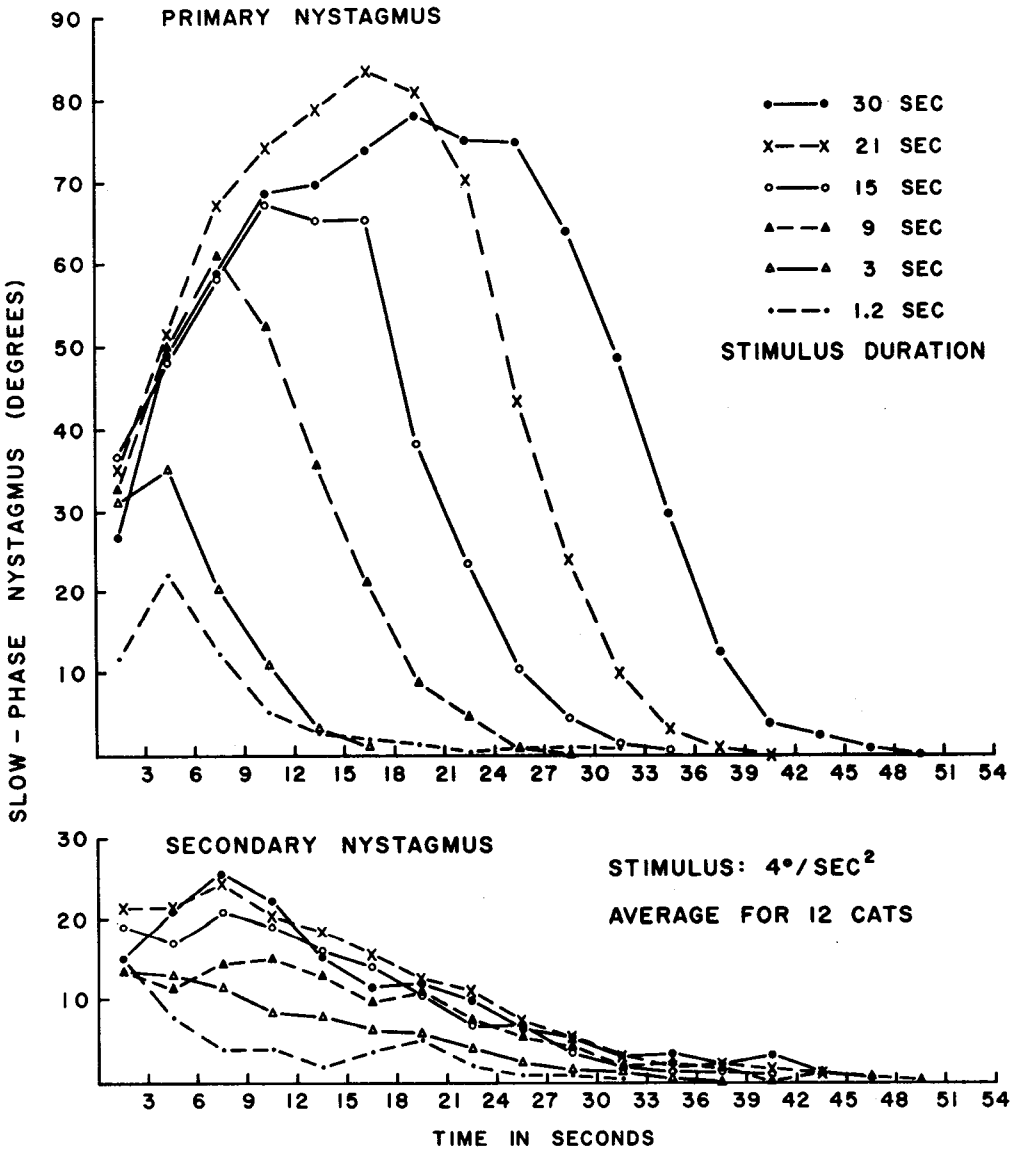

FIGURE 1. Time-course plots of slow-phase eye displacement per 3-sec interval for 12 cats exposed to 6 stimulus durations of 4°/sec² angular accelerations. For the 21 and 30 sec durations, responses reach a peak and begin to decline during the stimulus. For the two shortest durations, the peak response occurs after stimulus termination. Peak magnitude of the secondary reactions appears related to stimulus duration.

TABLE 2. Measures of primary nystagmus following the termination of each rotatory stimulus for cats. Each response value is a mean of responses to an acceleration and a deceleration stimulus. Stimuli were 4°/sec² for 1.2, 3, 9, 15, 21, or 30 seconds.

Time From End of Stimulus to End of Primary Nystagmus (Sec)

Cat	*1.2*	*3.0*	*9.0*	*15.0*	*21.0*	*30.0*
108	10.9	12.4	14.6	10.6	8.1	6.0
109	5.9	8.3	8.2	5.4	4.0	1.6
110	18.5	7.3	9.2	12.0	5.0	2.3
111	7.1	11.1	8.8	6.5	4.3	7.2
112	3.0	12.4	11.6	15.7	12.2	10.8
113	2.8	11.8	9.8	9.4	6.0	3.8
114	8.2	11.6	9.3	8.0	13.6	6.8
115	10.2	8.0	10.2	10.6	13.1	9.0
116	11.5	12.1	14.2	15.3	12.4	11.3
117	7.6	3.0	8.2	9.3	6.2	5.0
118	8.9	12.3	13.3	13.3	12.5	12.1
119	10.4	10.7	7.2	5.0	7.2	2.5
M=	8.8	10.1	10.4	10.1	8.7	6.5

Time From End of Stimulus to Start of Secondary Nystagmus (Sec)

108	15.6	15.4	20.8	15.6	14.5	13.2
109	7.9	16.9	13.5	9.9	6.5	6.2
110	15.9	6.6	12.9	6.0	8.4	6.7
111	15.0	16.8	12.6	10.8	7.5	10.6
112	——	14.6	17.5	24.4	20.4	18.1
113	15.7	18.3	14.1	11.6	9.0	7.5
114	12.5	12.7	14.7	25.4	15.4	15.6
115	17.3	19.5	14.6	16.7	15.0	14.0
116	13.6	16.9	21.3	17.5	15.4	16.1
117	11.2	18.0	14.8	12.6	13.0	15.6
118	9.3	16.3	15.3	15.5	16.4	15.7
119	15.2	14.9	11.8	11.4	9.5	7.1
M=	13.6	15.6	15.3	14.8	12.6	12.2

Beats of Primary Nystagmus After End of Stimulus

108	2.0	12.0	19.0	12.5	11.5	6.5
109	1.5	7.0	9.0	6.5	2.5	1.0
110	3.0	3.0	6.5	6.5	4.0	3.0
111	2.5	7.0	8.5	7.0	4.0	2.0
112	2.5	8.0	14.0	18.5	16.5	9.5
113	2.0	7.5	9.5	9.0	5.0	3.0
114	5.5	8.5	12.0	7.5	17.0	5.5
115	4.5	7.0	8.5	9.5	16.5	10.5
116	5.5	8.0	16.0	18.0	9.5	18.5
117	4.0	5.0	——	8.5	5.0	12.0
118	12.0	15.5	17.0	19.5	19.0	15.0
119	4.0	8.0	5.5	4.5	7.0	1.5
M=	4.1	8.0	11.4	10.6	9.8	7.3

TABLE 3. Measures of primary nystagmus following the termination of each rotatory stimulus for human subjects. Each response value is a mean of responses to an acceleration and a deceleration stimulus. Stimuli were 4°/sec² for 1.2, 3, 9, 15, 21, or 30 seconds.

Time From End of Stimulus to End of Primary Nystagmus (Sec)

Subject	*1.2*	*3.0*	*9.0*	*15.0*	*21.0*	*30.0*
Pa	22.0	11.0	17.3	32.0	16.8	22.3
Da	12.6	25.2	42.4	54.1	64.6	45.3
Br	——	28.8	20.5	25.7	37.5	29.6
Do	18.3	19.6	38.9	51.3	36.3	39.8
Ch	13.1	25.1	40.0	33.0	24.5	23.9
Ro	13.6	41.3	28.4	34.9	44.2	40.5
Ve	10.9	62.2	44.0	41.3	38.4	37.9
Te	——	20.9	42.6	26.7	33.1	27.2
Dt	31.8	42.0	44.4	48.4	40.1	35.7
Sa	21.3	22.3	55.6	44.1	32.8	28.8
Pz	7.3	29.1	57.9	40.3	39.2	28.5
Dy	17.6	46.3	42.4	45.6	33.7	29.2
M=	16.9	31.2	39.5	39.8	36.8	32.4

Time From End of Stimulus to Start of Secondary Nystagmus (Sec)

Pa	——	——	——	——	——	26.1
Da	——	——	——	——	——	67.3
Br	——	——	——	——	——	——
Do	——	——	——	——	——	——
Ch	——	——	30.6	42.8	28.3	27.1
Ro	——	——	72.7	41.3	51.4	45.4
Ve	——	——	48.8	46.1	40.2	38.1
Te	——	11.4	23.0	17.1	34.1	35.9
Dt	31.8	40.2	44.4	58.8	40.1	35.7
Sa	21.3	——	——	32.2	32.9	28.8
Pz	——	——	57.7	41.9	39.2	35.3
Dy	——	——	50.4	50.0	33.7	31.9
M=	*	*	46.8	41.3	37.5	37.2

Beats of Primary Nystagmus After End of Stimulus

Pa	13.0	13.0	19.5	36.5	39.0	27.5
Da	10.0	25.0	66.5	82.5	94.0	81.0
Br	——	27.0	11.5	29.0	33.0	29.5
Do	9.0	13.0	39.5	47.0	42.5	39.0
Ch	7.5	29.5	47.0	45.5	42.5	37.0
Ro	8.5	24.5	43.0	50.5	70.0	62.5
Ve	4.5	30.0	41.0	44.0	37.0	32.5
Te	——	23.0	53.0	46.0	51.5	38.5
Dt	12.0	39.5	55.5	63.5	59.5	55.5
Sa	20.0	31.5	78.5	73.0	52.5	54.0
Pz	4.0	12.0	34.5	34.0	28.5	24.0
Dy	15.0	20.5	47.0	51.0	46.5	43.5
M=	10.4	24.0	44.7	50.2	49.7	43.7

*Too few scores on which to base a mean.

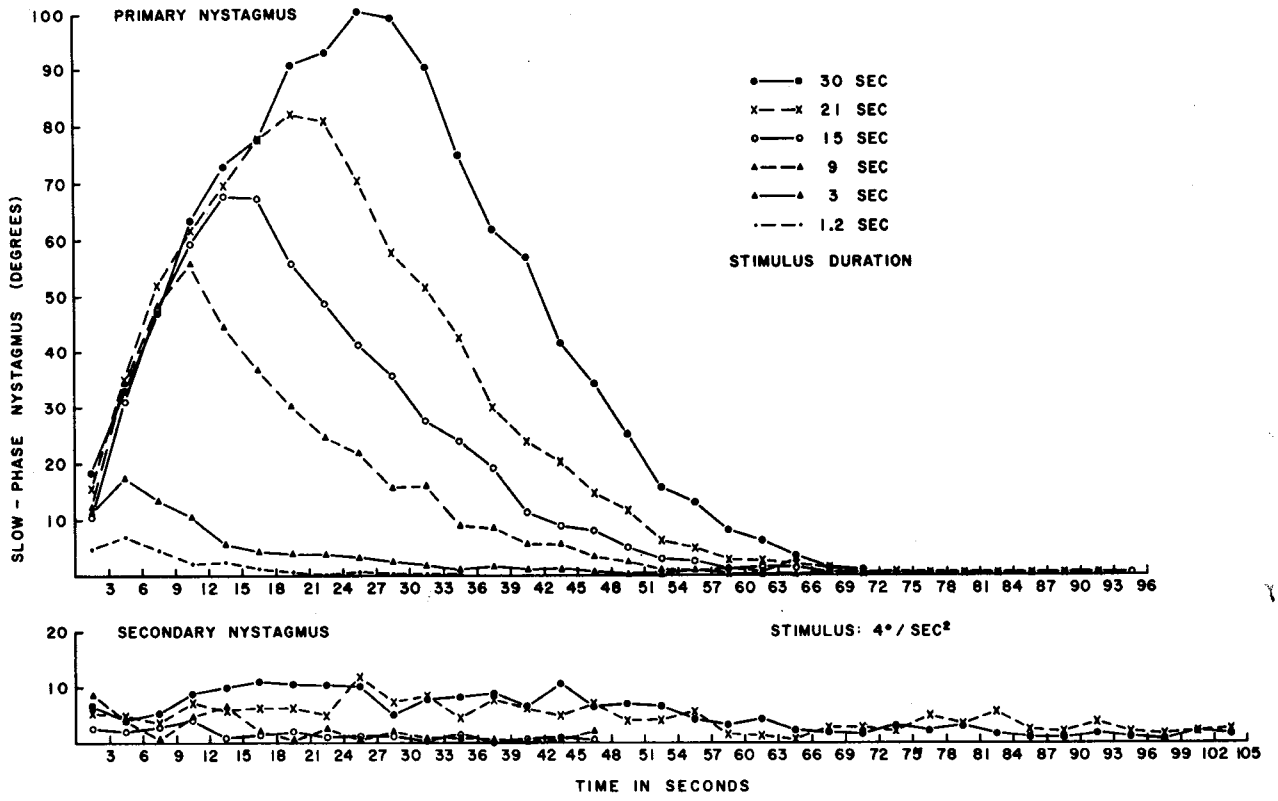

FIGURE 2. Time-course plots of slow-phase displacement per 3-sec interval for 12 human subjects exposed to 6 stimulus durations of 4°/sec² angular accelerations. No decline of nystagmus during stimulation is evident for any duration (compare with Figure 1). Note that the peak response for the three shortest durations occurs after stimulus termination. No secondary responses were evident for the 1.2 and 3 sec stimuli and not all subjects showed secondary nystagmus for the longer stimuli.

The slow-phase velocity of primary and secondary nystagmic responses was plotted for each of the 6 stimulus durations (Figure 1). For the 21 and 30 sec stimuli, there was a slight decline in response during constant angular acceleration. Consistent with data from cats in the earlier experiment,[6] it appears that the nystagmic response reaches maximum intensity between 15 and 20 sec and declines after this, even though the stimulus is of constant magnitude and direction.

The peak magnitude of secondary nystagmus appears directly related to the duration of the angular acceleration. Secondary responses were obtained from all but one cat for the 6 stimulus-duration conditions; the exception (cat 112) gave no secondary nystagmus following the 1.2 second stimulus.

B. Human Subjects

Figure 2 shows little or no decline in human nystagmus during constant angular acceleration irrespective of stimulus duration. This is consistent with human results of earlier studies,[5,6,14] but it is in contrast with results from cats in which nystagmus declined after about 20 sec of constant angular acceleration.

With brief stimuli (1.2 sec and 3 sec), it appears that in both man and cat the slow-phase velocity of nystagmus continues to increase ("overshoots") after the stimulus terminates (Figures 1 and 2), whereas, with prolonged stimuli, slow-phase velocity of nystagmus declines immediately after (in humans) or before (in cats) the stimulus terminates (Figure 1).

As stimulus duration is increased up to about 15 seconds, the duration of the post-stimulus primary nystagmus increases. With longer stimuli, the duration of the post-stimulus primary response declines. In this respect human primary nystagmus corresponds fairly well in its temporal characteristics with the human subjective response. This is shown in Figure 3

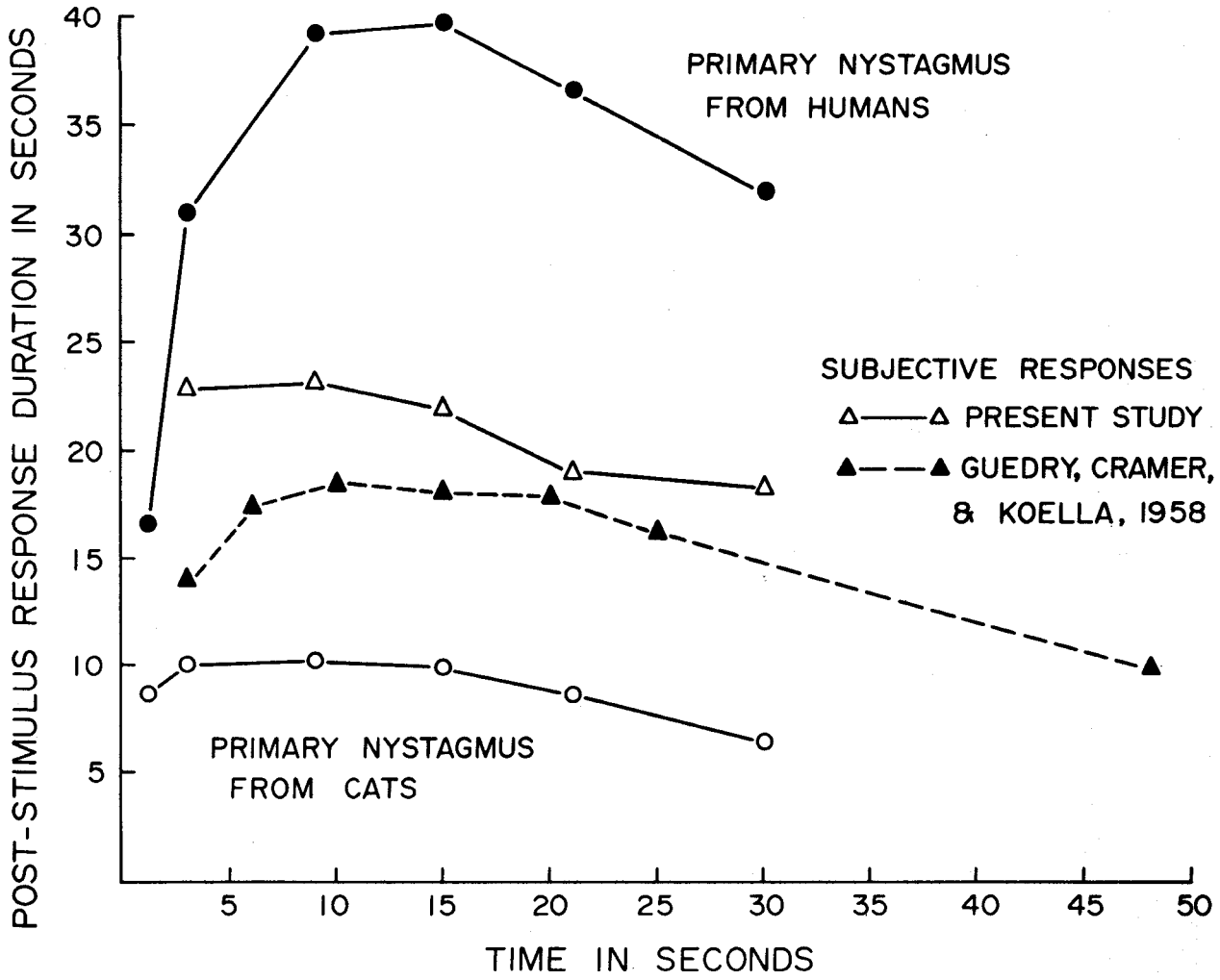

FIGURE 3. Duration of the post-stimulus subjective and nystagmic reactions obtained in this study are compared with subjective data from an earlier study. The abcissa represents stimulus duration. Functions depicted for the two sets of subjective data and for primary (slow-phase displacement) nystagmus from cats are in close agreement.

where the mean subjective turning scores from the present study are presented along with subjective data obtained in a previous study. In this earlier study,[13] subjects had been trained in making subjective reports, whereas the present subjects had no preliminary practice. The rise and decline in duration of human nystagmus and subjective response as stimulus duration increases is also matched fairly well by the change in temporal characteristics of nystagmus in the cat, also shown in Figure 3. Reference to Tables 2 and 3 shows that, in both man and cat, time elapsed from stimulus termination to onset of secondary nystagmus increases and declines in a manner which approximately parallels duration of the primary reaction.

Secondary nystagmus was not evident in the recordings of any of the human subjects for the 1.2 and 3 sec stimuli, and several subjects gave no secondary response to the 30 sec stimulus. However, frequency of occurrence of secondary nystagmus increased with stimulus duration, and this may be interpreted as evidence for a relationship between stimulus duration and intensity of secondary response in humans similar to, but more variable than, that observed in the cat. In comparison with secondary nystagmus of the cat, the secondary nystagmus in man has a later onset, lower average intensity (relative to man's primary reaction), and seems to be more subject to individual differences.

Tables 2 and 3 suggest that the number of

beats of secondary nystagmus is more closely related to the duration of primary post-stimulus nystagmus than to the duration of the stimulus. This is in contrast to the maximum slow-phase velocity of secondary nystagmus which seems related to the stimulus duration, at least in the cat (see Figure 1). The decline in number of secondary beats with stimuli longer than 15 sec may signify an encroachment of the secondary reaction on the primary reaction.

Primary nystagmus in the cat is shorter in duration and has a lower beat-frequency than that of man for the range of stimuli investigated. There is also a pronounced difference between man and cat in regard to the intensity-ratio of secondary to primary nystagmus, the ratio being higher for the cat.

A log plot of nystagmus slow-phase velocity with respect to time also shows the cat to have a substantially different rate of decline of nystagmus (lower time constant) than man for comparable stimuli (see Figure 4). It also appears that the slope of nystagmus decline for both man and cat changes as a function of stimulus duration, the longer stimuli producing post-stimulus nystagmus with a higher rate of decay (lower time constant).

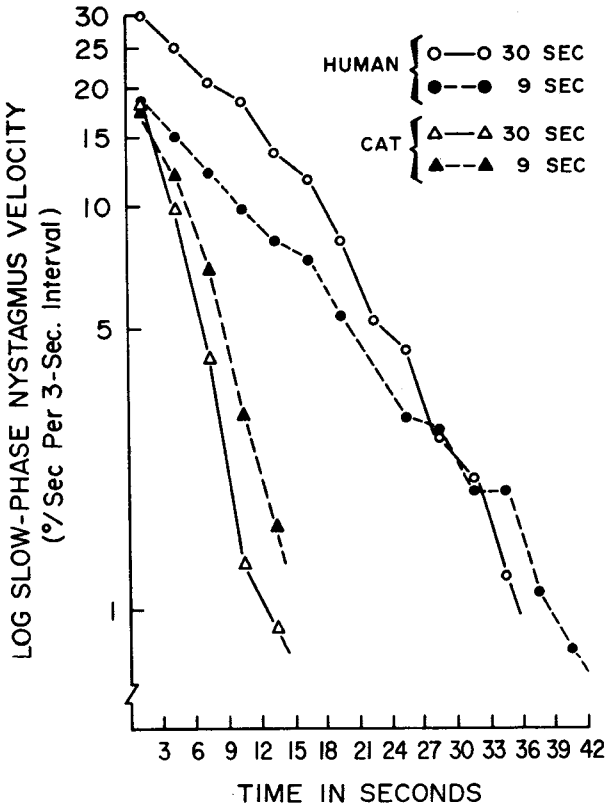

FIGURE 4. Log plots of slow-phase eye velocity following two stimulated durations for cat and human subjects. Data represent nystagmus measured from the point of stimulus termination and thus depict the rates of response decay. Decay is more rapid in cats and the longer stimulus duration produces more rapid declines.

V. General Discussion

Because neurophysiological data from cats are sometimes applied to the explanation of reactions in man, it is important to compare the same response variables in men and cats exposed to identical vestibular stimuli. Nystagmus in cats declined during prolonged constant angular acceleration in spite of efforts to maintain alertness, whereas nystagmus in alert men did not decline during prolonged angular acceleration; the rate of decline of primary vestibular nystagmus following each stimulus was greater in cats than in man; the ratio of secondary to primary nystagmus was higher in cat than in man.

If it is assumed that secondary nystagmus reflects a process which develops during the primary reaction and opposes its continuation, then all of the differences between man and cat may be due to this secondary process in cat having a more direct control of nystagmus than does its counterpart in man. The characteristics of subjective data reported by man resembled the alterations of nystagmus in cats during and after prolonged angular accelerations,[6] and in some respects the nystagmus of man deviates from both the nystagmus of cat and the subjective responses of man during and after these unusual stimuli. It appears that the hypothesized secondary process exerts a control on man's sensory experience, i.e., the perceived angular velocity, comparable to the control of nystagmus in the cat. This is not to say that the nystagmus of man is completely without the modulating influence of this secondary process because the rate of decline of both post-stimulus primary nystagmus and the occurrence of secondary nystagmus were influenced in man by the duration of the stimulus. However, the control is apparently less consistent and, hence, probably less direct for the range of stimuli used in the present experiment. (Subsequent experiments have illustrated a pronounced secondary nystagmus in

man when prolonged high-magnitude angular accelerations are used.)

It is parsimonious and reasonable to assume that a single process accounts for (a) the decline in response during prolonged angular acceleration, (b) the increased response decline following prolonged acceleration, and (c) the increased secondary response following prolonged acceleration. The possibility that the cupula endolymph system is under-critically damped, contrary to the common supposition, would explain a secondary response, but this seems at variance with much of the evidence for the over-damped Torsion Pendulum analogy of van Egmond, Groen, and Jongkees[21] and could not explain findings (a) and (b). To explain these results on the basis of cupula deflection, it would be necessary to assume that the stiffness of the cupula increases during prolonged constant angular acceleration. Van Egmond, et al[21] have proposed that events within the endorgan (either bioelectric, biochemical or plastic changes within the cupula) could account for secondary responses, and this possibility cannot be ruled out. Lowenstein[16] has reported that increasing cupula deflection in elasmobranch initiates neural activity "in one after the other previously silent units" which often adapt themselves rapidly. Adrian[1] and recently Melvill Jones,[17] recording from the vestibular nuclei in cats, found little evidence for rapid adaptation. However, Cappel[3] has noted that slow declines in some units recorded in the vestibular nuclei of cats correspond temporally to declines in human subjective data. The possibility that there is a diminished sensory inflow during prolonged cupula deflection in cats remains open.

In man, one aspect of the vestibular reaction (subjective velocity) declines while another aspect (nystagmus) does not decline during prolonged acceleration.[14] From this, it does not seem reasonable to attribute the decline of the one response to a suppression of sensory inflow. (Some reservations are necessary in this interpretation because average curves of different groups of subjects form the basis of the conclusion. Subjective and nystagmic data from the same subjects should be compared and studied for the presence of correlation.) Aschan and Bergstedt[2] have evidence which implicates the central nervous system in secondary responses. Prolonged primary responses induced by unilateral caloric stimulation should provide an opportunity for adaptive changes within the cupula; yet Aschan and Bergstedt[2] reported little or no secondary nystagmus with unilateral caloric stimuli, whereas bilateral hot/cold caloric stimuli and rotational stimuli, yielding primary responses of equivalent length, produced secondary responses.

Although secondary nystagmus (and apparently associated response modulation) in man and cat are probably attributable to the central nervous system, it remains quite possible that some of the differences between man and cat are attributable to differences in the properties of endorgans; e.g., the shorter primary nystagmic response in cats (irrespective of stimulus duration) is probably attributable to a greater cupula spring-action in cat. Response parameters of the central nervous system may be conditioned or inherently matched to the response parameters of the cupula-endolymph system so that the shaping of responses to unusual stimuli may be similar in form but on a different time base in different animals.

The conditions under which the "secondary processes" have been demonstrated, viz., prolonged constant angular acceleration, are seldom, if ever, encountered in natural movement. Even a single, brief, unidirectional angular acceleration followed by constant velocity does not occur in natural movement and, as noted in the present study, intensity of nystagmus continues to increase briefly beyond the termination of short unidirectional stimuli. In natural movement, any brief angular acceleration is immediately followed by angular acceleration of opposite sign which returns the cupula toward its position of static equilibrium. Hence, in the case of either brief unidirectional stimuli or prolonged unidirectional stimuli, the vestibular reactions fail to follow the theoretical[11] cupula deflection. However, this does not necessarily signify either an inadequate response system within the range of natural movements or a gross error in theoretical cupula mechanics. The departure from expected results signifies a range of unnatural stimuli which is not accurately followed due to either inaccurate sensory detection or unfaithful central following of the input, or both. Because the natural periods of movements of various animals are different,[18] it is quite possible that the ranges of accurate sensory

representation of movement, due to central and peripheral differences, will differ slightly in different animals.

The functional significance of the secondary process is not established. Some pathological conditions undoubtedly yield a central imbalance of spontaneous input from the two ears, and the "secondary process" may serve to readjust the point of homeostatic balance. Some complex motions of the head and body may terminate with minor residual cupula deflections, and this could require minor shifts in the point of balance between the two ears, which would be accomplished too slowly by the elasticity of the cupula. Tolerance to an increased level of vestibular stimulation encountered in land, sea, and air travel may require a suppression at some level of vestibular inflow, and it is possible that this secondary process serves this function. It has been shown that standard test stimuli administered after an angular acceleration are influenced in proportion to the duration of the preceding angular acceleration,[13] and it may be assumed that this finding is another manifestation of the "*secondary process.*" Moreover, with repetitive angular accelerations, the peaks of both primary and secondary nystagmus in cats diminish and shift toward earlier occurrences.[4,6] This suggests that the secondary process encroaches more and more upon the primary reaction and thus limits the magnitude and duration of the primary reaction with repetitive stimulation. In humans, there are large individual differences in secondary nystagmus. If the secondary reaction is a manifestation of an adaptive process which serves to limit the primary reaction, it may prove to be an indicator, among people with comparable histories of motion exposure, of individual differences in ability to habituate to repetitive vestibular stimulation.

VI. Summary

A direct relationship between duration of acceleration and (a) decline of response during acceleration, (b) rate of decline of response after acceleration, and (c) magnitude of secondary reaction, is regarded as an indication of a central process which limits a prolonged vestibular primary reaction. The process is manifested by its influence on relatively basic reflex reactions (nystagmus) in the cat, and is more prominently manifested in man by its influence on sensory perception.

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DIAGNOSTIC TESTS OF COLOR DEFECTIVE VISION

Annotated Bibliography, 1956–66

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Office of Aviation Medicine

FOREWORD

This annotated bibliography of recent literature on diagnostic tests for color defect is presented as a scientific service of the Civil Aeromedical Institute. The aims of the publication are: (a) to provide interested researchers with source material and (b) to provide medical examiners with material relevant to the diagnosis of color defect.

In achieving these aims, bibliographic listings such as the present one are necessarily limited in scope. However the survey is not limited to the years listed. Papers from years prior to 1956 concerning tests that are currently available commercially have been included and one reference is included from 1967. No attempt is made to evaluate the scientific worth of a given article.

References 23, 24, and 83 have recently been translated. Copies of the translations of these papers only may be obtained by writing to: Medical Librarian, HQ–640, Federal Aviation Administration, Washington, D.C. 20553. Please provide the librarian with the complete reference information.

DIAGNOSTIC TESTS OF COLOR DEFECTIVE VISION

Annotated Bibliography, 1956–1966

1. BAILEY, R. W. Color vision deficiencies in army fliers. USAARU Report No. 65–2. April, 1965, Fort Rucker, Alabama.

   This paper deals with a review of some color tests and a testing procedure employed to determine the number of color anomalous fliers in army aviation. Data collected indicate that the color requirements may be unnecessary and that a new approach is overdue.

2. BAKER, H. D. Single-variable anomaloscope matches during recovery from artificial red blindness. *J. Opt. Soc. Amer.*, 56:686–689, 1966.

   An anomaloscope was designed so that full amounts of its red and green primaries could be adjusted to match yellow.

3. BARON, J., CABAU, A., & PARMENTIER-BELOUX, M. Application du test de Farnsworth 100 hue dans l'examen de la vision colorée. *Le Presse Med.*, 64:561–562, 1956.

   The Farnsworth 100 Hue test was examined and compared with other tests. The authors concluded that the Farnsworth 100 Hue test was good for diagnosing the anomalies of color vision and also for diagnosing difficulties in color discrimination.

4. BELCHER, S. J., GREENSHIELDS, K. W., & WRIGHT, W. D. Color vision survey using the Ishihara, Dvorine, Boström, Boström-Kugelberg, and American-Optical Hardy-Rand-Rittler tests. *Brit. J. Ophthal.*, 42:355–359, 1958.

   Five hundred subjects were tested with five different sets of pseudo-isochromatic plates. The tests were compared for division of subjects between normal and defective and for screening efficiency of each plate.

5. BOYNTON, R. M. & WAGNER, M. Two-color threshold as a test of color vision. *J. Opt. Soc. Amer.*, 51:429–440, 1961.

   The two-color threshold is obtained by finding the just visible luminance of a test flash of one color seen against the background of another color. An examination of green-on-red thresholds plotted against red-on-green thresholds reveals a separation of normal subjects from the color defective. Within the color defective group, the test seems to provide a quantitative assessment of the degree of color defect.

6. BURNHAM, R. W. & CLARK, J. R. A color memory test. *J. Opt. Soc. Amer.*, 44:658–659, 1954.

   A test for color memory has been developed using chips from the Farnsworth-Munsell hue series. The observer selects from a hue circle of color samples the one which most resembles a test sample presented a short time before. This procedure is repeated for a number of test samples.

7. CAMERON, R. G. Rational approach to color vision testing. *Aerospace Med.*, 38:51–59, 1967.

   A rational approach to color vision testing demands the elucidation of three points: whether a color vision defect is actually present, what it is, and whether the defect is compatible with the requirements of the examining service. Testing of 5,141 pilot candidates indicates 5.17% defectives. This indicates that about 2½% of the defectives were self-eliminated, and the rejection of 3.9% of all candidates can be regarded as an average figure. The paper offers a plea for uniformity of definition, of methodology, and particularly of terminology.

8. CHAPANIS, A. A comparative study of five tests of color vision. *J. Opt. Soc. Amer.*, 38:626–649, 1948.

   This study compares the American Optical Company, Ishihara, Meyrowitz, Boström, and Boström-Kugelberg color vision tests. The author reports that the Boström-Kugelberg test is the best all around test of color deficiency evaluated in the study. The Ishihara was next best. The author found the Bostrom and Meyrowitz plates to be unsatisfactory. The author reports that neither visual acuity nor age is related to performance on the tests used in this study.

9. CHAPANIS, A. Diagnosing types of color deficiency by means of pseudo-isochromatic tests. *J. Opt. Soc. Amer.*, 39:242–249, 1949.

   Five pseudo-isochromatic tests of color vision (the American Optical Company, Ishihara, Meyrowitz, Boström, and Boström-Kugelberg) were evaluated for their ability to differentiate two kinds of color deficient individuals. The Bostrom plates were found to be worthless for this purpose, the Meyrowitz plates somewhat better, and the Ishihara plates best. None of the plates in the other two tests was found to be diagnostic.

10. COLE, B. L. Misuse of the Ishihara test for color blindness. *Brit. J. Physiol. Opt.*, 20:113–118, 1963.

    This paper examined the weaknesses of the Ishihara test for color blindness and suggested two working rules for its use: (1) A patient who misreads three plates or less is almost certain to have normal color vision. If he misreads between three and six plates, he can be considered to be normal, provided some of the errors are not typical of the errors that the color defective makes. (2) No reliable quantitative assessment of the severity of the defect can be ex-

pected from the Ishihara test although between six and fifteen errors may indicate a mild defect.

11. COLLINS, W. E., CASOLA, A. S., & ZEGERS, R. T. The performance of color-blind subjects on the color aptitude test. *J. Gen. Psychol.*, 64:245–250, 1961.
The paper compared the performance of color deficient subjects on the Color Aptitude test with performance on the Hardy-Rand-Rittler plates. The results show that the color defectives had poor scores on all color series of the Color Aptitude test. There was some evidence that lower scores on the red series may indicate a protan-defect, while lower scores on the green series may indicate a deutan-defect.
12. COLOMBI, A., VITALI, G., & CARTA, F. Impiego di una funzime discriminante nella valutazime della capacta diagnostica delle favole di Ishihara. *Annal. Ottal. Clin. Ocul.*, 91:954–961, 1965.
This paper described the use of a discriminative function in the evaluation of the diagnostic efficiency of the Ishihara plates.
13. CRAWFORD, A. A description of an anomaloscope. *Brit. J. Physiol. Opt.*, 8:173–175, 1951.
This paper described a simple filter anomaloscope.
14. CRAWFORD, A. The Dvorine pseudo-isochromatic plates. *Brit. J. Psychol.*, 46:139–143, 1955.
This is a review of the second edition of the Dvorine pseudo-isochromatic test. The reviewer concluded that the test is an effective screening test for separating color defectives from color normals.
15. CRONE, R. A. Clinical study of color vision. *Brit. J. Ophthal.*, 39:170–173, 1955.
This paper described a modification of the Nagel anomaloscope, which permitted determination of (1) relative luminous efficiency of spectral light, (2) thresholds of wavelength discrimination in the spectrum, (3) thresholds of saturation discrimination in the spectrum, and (4) trichromatic coeffiicients of spectral colors.
16. CRONE, R. A. Quantitative diagnosis of defective color vision: A comparative evaluation of the Ishihara test, the Farnsworth dichotomous test, and the Hardy-Rand-Rittler polychromatic plates. *Amer. J. Ophthal.*, 51:298–305, 1961.
The paper compared the pseudo-isochromatic plates of Ishihara with the Hardy-Rand-Rittler plates and the Farnsworth Dichotomous test. The threshold of wavelength discrimination at 590nm was taken as a measuring rule. The Ishihara plates proved excellent for differentiation between normals and abnormals but were unsuitable for quantitative differentiation. The H-R-R plates, on the contrary, were unsuitable for differentiating between normals and abnormals, but were very good for quantitative differentiation. For the latter, the Farnsworth Dichotomous test was also usable though to a lesser degree.
17. DIMMICK, R. L. & WIENKE, R. E. How red is red? *Amer. J. Psychol.*, 71:298–304, 1958.
Selected portions of the red end of the spectrum were evaluated. The results indicated that the use of different primaries in anomaloscopes will result in slightly different red-green ratios. The results indicated that the red-green ratio is a function of the luminance level of the yellow comparison stimulus. The authors suggested that more rigorous results may be obtained in anomaloscopic examinations if the yellow comparison stimulus is at a fixed luminance level.
18. DVORINE, I. Quantitative classification of the color blind. *J. Gen. Psychol.*, 68:255–265, 1963.
A comparative study of 34 color vision defectives disclosed that 19 of them were diagnosed alike with the Dvorine, H-R-R, and Tokyo Medical College tests; the Dvorine and TMC tests were in agreement quantitatively in 27 of 34 cases; the Dvorine and the H-R-R were in agreement quantitatively in 25 of 34 patients; and the H-R-R and TMC were in agreement quantitatively in 20 of 34 cases presented. The author concluded that failure to pass a color vision test which is based on progressive increase of chroma in polychromatic plates (H-R-R and TMC) would seem less effective an indicator of the extent of color vision defect than the estimation of the deficiency from the number of missed plates or errors on the Dvorine test.
19. DVORINE, I. The importance of color vision in the military services. *Optom. Wkly.*, 54:1191–1195, 1963.
The author described the various types of color vision tests in use. He recommended the pseudo-isochromatic test above all other types, but suggested that the armed services must have their own set of plates designed for their exclusive use.
20. FARNSWORTH, D. The Farnsworth-Munsell 100 hue and dichotomous tests for color vision. *J. Opt. Soc. Amer.*, 33:568–578, 1943.
This paper described the construction and use of the Farnsworth-Munsell 100 Hue test and the Farnsworth Dichotomous test for color vision.
21. FARNSWORTH, D. Testing for color deficiency in industry. *A.M.A. Archs. Ind. Hlth.*, 16:100–103, 1957.
A four-part classification of color vision was suggested based upon whether applicants pass or fail pseudo-isochromatic plates, the Farnsworth Lantern, or the Farnsworth D–15 Dichotomous test. An applicant who passed all three tests was described as normal. An applicant who passed the lantern and the Dichotomous test but failed the plates was classified as a mild defective. An applicant who passed the Dichotomous test but failed the plates and the lantern was described as moderate defective. An applicant who failed all three tests was classified as a severe defective.
22. FARNSWORTH, D. & FOREMAN, P. Development and trial of the New London Navy lantern as a selection test for serviceable color vision. BuMed Project X–457 (AV–241–K), Color Vision Rpt. No. 12, 6 May 1946. U.S. Submarine Base, New London, Connecticut.
A color vision testing lantern was designed which was intended to be as quick and convenient to give as pseudo-isochromatic plates or other standard

tests, which would be more reliable in its pass-fail criteria and less dependent upon the training and personal interpretation of the examiner. A model of the proposed Navy Lantern, called the New London Prototype Model, was tested on over 2,000 individuals and compared with other standard tests for color vision.

23. FLEKKEL, A. B. Differential diagnosis of color vision. (in Russian) *Dokl. Akad. Nauk. SSSR.*, 100:57–60, 1955.
This paper compared the performance of color defectives on the Rabkin plates and on the Rautian anomaloscope in distinguishing color signal lights. The paper concluded that Rayleigh equations are an inadequate index of color defect.

24. FLEKKEL, A. B. An experiment in the application of Yustova's tables for investigation of color vision. (in Russian) *Biofizika*, 1:708–712, 1956.
This paper described the Yustova plates for color vision testing and their use.

25. FREY, R. G. Welche pseudoisochromatischen Tafeln sind für die Praxis am besten geeignet? *Albrecht v. Graefes Arch. Ophthal.*, 160:301–320, 1958.
This paper compared the Boström, Boström-Kugelberg, H-R-R, Ishihara, Rabkin, and Stilling color plate tests.

26. FREY, R. G. Zur Vereinfachung der Farbensinnprüfung. *Wien. Klin. Wschr.*, 73:852–854, 1961.
In order to reduce the time spent in testing color vision and, at the same time, to make reliable tests, it is necessary to combine pseudo-isochromatic plates with high selectivity and usefulness. The author recommended use of the following four plates: Farnsworth's tritan plate, Boström's plate 10, Bostrom-Kugelberg's plate R, and Rabkin's plate 14.

27. FREY, R. G. Die Trennschärfe einiger pseudoisochromatischer Tafelproben. *Albrecht v. Graefes Arch. Ophthal.*, 165:20–30, 1962.
This paper compared the pseudoisochromatic plate tests of Dvorine, Velhagen, and Polack. The author recommended the Dvorine without changes. He concluded that the Velhagen test becomes more useful when restricted to the 17 plates of raised selectivity. He concluded that a selection of only 14 plates from the Polack test is useful.

28. FREY, R. G. Zur Differentialdiagnose der angeborenen Farbensinnstörungen mit pseudoisochromatischen Tafeln. *Ophthalmologica (Basel)*, 145:34–48, 1963.
A collection of pseudo-isochromatic plates was examined to determine their differential diagnostic possibilities and the results were compared with anomaloscope findings. It was possible to a certain extent to determine whether a protan or deutan disturbance existed. The Rabkin tables produced a correct qualitative diagnosis in 97.4% of the cases, Dvorine in 94%, Hardy-Rand-Rittler in 90.1%, Polack in 88%, Ishihara in 82.7% and the Tokyo Medical College color vision test in 65.2%. The quantitative diagnosis in all cases varied considerably from the findings of the anomaloscope. More particularly those cases which would be classified as "mild" by the Hardy-Rand-Rittler tables or by the Tokyo Medical College test should probably be classified as anomalous trichromats. From examinations made with the TMC no certain conclusions can be drawn as to the nature and extent of the color disturbance.

29. FREY, R. G. Zur Wahl der geeigneten Beleuchtung für die Farbensinnprufung mit pseudoisochromatischen Tafeln. *Wien. Klin. Wschr.*, 76:170–172, 1964.
This paper enumerated the illumination and color temperature of the illuminants required by various pseudo-isochromatic plates. The author emphasized the need for holding illumination constant in the testing of subjects.

30. FREY, R. G. Erfahrungen mit der 7. Auflage der polychromatischen Tafeln J. B. Rabkins. (Experiences with the seventh edition of the J. B. Rabkin polychromatic tables). *Klin. Mbl. Augenheilk*, 145:97–106, 1964.
The seventh edition of the "Polychromatic charts" of Rabkin were used to test two groups of 170 persons each, who had been examined with the anomaloscope and classified accordingly into one group with normal color sense and another with defective color sense. In an abridged form, this collection can be recommended for the rapid and reliable detection of persons with defective color vision. A positive differential diagnosis cannot be established by means of these charts. The charts for the examination of the color vision of children, charts which were enclosed for the first time in this edition, need to be improved.

31. GALLAGHER, J. R. & GALLAGHER, C. D. Color vision screening of preschool and first grade children. *Arch. Ophthal.*, 72:200–211, 1964.
Observations relevant to the color vision testing of preschool and first grade children were reported. About 3% of each of the two groups studied appeared to have a color vision defect of sufficient degree to constitute a practical handicap in correctly discriminating colors. A simple screening method for detecting significant degrees of color vision deficiency in preschool and first grade children was described. It is based on a selected number of AO H-R-R plates.

32. GOLDSTEIN, A. G. & BROOKS, R. A red-green color vision test employing transparencies. *Percept. Motor Skills*, 11:229–230, 1960.
A red-green color vision test employing transparencies was described and data for 18 subjects were presented. The authors recommended continuing research to determine whether correctly exposed transparencies may be employed for group color vision testing.

33. HAEFNER, R. A decade of research in color blindness. *Psychol. Newsltr., N.Y.U.*, 8:128–148, 1957.
This is a survey article reviewing the literature on color defects from 1946 to 1955, inclusive. All aspects of color defect, including color testing, were reviewed.

34. HARDY, L. H., RAND, R., & RITTLER, M. C. Test for the detection and analysis of color blindness. III. Rabkin test. *J. Opt. Soc. Amer.*, 35:481–491, 1945.
From an examination of the second edition of the Rabkin test the authors concluded that, properly administered, the test affords a good device for screening the color defective from the color normal if 75 is taken as the critical performance score. The test taken as a whole affords an excellent means of classifying red-green dichromats into the two groups: protanopic and deuteranopic; it also provides for classification of red-green anomalous trichromats into two groups: deuteranomalous and protanomalous. The test is not adequate to differentiate between anomalous trichromasy and dichromasy.

35. HARDY, L. H., RAND, G., & RITTLER, M. C. The H-R-R polychromatic plates. I. A test for the detection, classification, and estimation of the degree of defective color vision. *A.M.A. Arch. Ophthal.*, 51:216–228, 1954.
The paper described the construction, administration, validation, and reliability of the H-R-R polychromatic plates.

36. HARDY, L. H., RAND, G., & RITTLER, M. C. The H-R-R polychromatic plates. II. Comparison of qualitative and quantitative classifications by H-R-R plates and other tests. *A.M.A. Arch. Ophthal.*, 52:353–368, 1954.
This paper presented further validation and reliability information concerning the H-R-R polychromatic plates. It presented a comparison of the qualitative and quantitative diagnoses yielded by the H-R-R plates with performances on other tests of color vision.

37. HARDY, L. H., RAND, G., & RITTLER, M. C. H-R-R polychromatic plates. *J. Opt. Soc. Amer.*, 44:509–523, 1954.
This paper presented approximately the same information as the two previous papers.

38. HIOKI, R. & NAKAMURA, Y. Hioki's polarization anomaloscope. *Amer. J. Ophthal.*, 40:559–562, 1955.
This paper described construction of a polarization version of the Nagel anomaloscope.

39. HOMBERG, L. The distribution and reliability of certain color vision data as measured by the Pickford-Nicolson anomaloscope. *Psychol. Res. Bull.*, 3:7pp., 1963.
This paper presented the standardization data for the Pickford-Nicolson anomaloscope. The instrument was found to be a valuable and sensitive tool for color vision testing as well as for psychophysical investigation.

40. HOOGERHEIDE, J. Considerations about the acceptability of mild color defective trichromats in flying personnel. *Aeromed. Acta*, 7:17–23, 1959, 1960.
The author recommended testing flying personnel with the Hardy-Rand-Rittler plates. He also recommended passing those people who make no mistakes in the medium and strong plates. The author suggested that pilots with mildly anomalous color vision do not pose any danger.

41. JOSHI, V. G. Brightness contrast as a source of error in the Ishihara test for color blindness. *Brit. J. Physiol. Opt.*, 18:239–242, 1961.
Plates of the Ishihara test for color blindness were photographed in black and white by so modifying the technique of exposure that the color sensitivity of the film emulsion was made uniformly constant to all spectral colors. The older the edition, the greater the visibility of the numerals in the photographs. Factors of "color contrast" and "brightness contrast" appeared to be of comparative importance in the Ishihara test.

42. KATAVISTO, M. Pseudo-isochromatic plates and artificial light. *Acta Ophthal. (Kobenhavn)*, 39:377–390, 1961.
Thirty persons with normal color vision and 50 with defective color vision were tested with Boström II, Ishihara, and Stilling pseudo-isochromatic plates in daylight, and by the light of tungsten lamps and flourescent tubes with high color temperature. Similar results were obtained with the Boström plates with each of these three sources of light, and with the Ishihara plates in daylight and in the light of flourescent tubes. The results obtained with Stilling plates were reliable only in daylight. Thus, the Bostrom and Ishihara plates are well suited for tests of color vision by light of flourescent tubes, which can be recommended as a standard illuminant during the dark winter months.

43. KATAVISTO, M. Vääriaistin tutkimisesta pseudoisokromaattisilla tauluilla. (Color sensitivity examination with pseudo-isochromatic tables). (in Finnish) *Suom. Lääklehti*, 17:121–126, 1962.
The principles for use of pseudo-isochromatic tables and the reliability of obtained results were surveyed by making comparisons between the Stilling, Boström (IIB), Ishihara, and Tokyo Medical College tests. The latter was recommended as a rapid screening test for mass examinations of color vision. The importance of an accurate sense of color in certain professions was stressed and, hence, it was proposed that the testing of color vision be included in vocational guidance.

44. KIRSCHEN, M. The color rator: a new instrument for the assessment of color aptitude in industry. *Amer. J. Optom.*, 36:137–143, 1959.
An instrument was described for the determination of color aptitude or color discrimination with the red and green portions of the spectrum. The matching principle was used. An additional advantage of this instrument was that the procedure as outlined was sufficiently simple for its administration by a lay person at the employment level of industry. Criteria for its use were proposed from a preliminary study of 21 subjects. Range of settings at various wavelengths was adopted as principle criterion for pass or fail decisions.

45. LAKOWSKI, R. Testing of colour vision in prospective printers' apprentices and the problems this presents in selection. *Brit. J. Physiol. Opt.*, 22:10–32, 1965.

The performance of the printers' apprentices (boys between the ages 14–17) on all the color vision tests used in this study was different from that of the control group. This difference appeared as differences in the most frequent type of response accepted by the members of each of the two populations, and as differences in the variability of such performances. This was best seen in the anomaloscope, where there was a difference in terms of mid-matching points accepted by the two groups and this applied also to variability of their matching ranges. There was a decidedly higher incidence of subjects with minor color deficiencies in the experimental group. There were twice as many deuteranopes as found in other studies. The author suggested that perhaps ages 14 through 16 are the best times to test color vision for vocational purposes, for at this stage any latent weakness will be indicated.

46. LINKSZ, A. The Farnsworth panel D–15 test. *Amer. J. Ophthal.*, 62 :27–37, 1966.
This paper discussed the principles and uses of the Farnsworth panel D–15 test. Extending its use for diagnosing tetartan and achromatic defects was also discussed.

47. MAJIMA, A., AWAYA, S., & TANABE, S. Evaluation on Ishihara plates (numeral and winding-line plates), H-R-R plates, and T.M.C. plates for screening tests. (in Japanese) *Jap J. Clin. Ophthal.*, 18 :493–496, 1964.
The authors reported that the Ishihara is an excellent test for screening, while the H-R-R plates proved unsatisfactory for this purpose.

48. MCCULLOCH, C., TURNOUR, N. C., & SMILEY, J. R. A field study of Hardy-Rand-Rittler color vision plate test. *Amer. J. Ophthal.*, 48 :124–129, 1959.
This paper compared the H-R-R color vision test with the American Optical Company pseudo-isochromatic plate test used by the RCAF and with the RCAF lantern. The authors suggested that with the Hardy-Rand-Rittler test a simplified form can be used and subjects may record their own responses.

49. MUNTEANU, M. Examination of the colour sense in the navy. The chromatoscope. *Rev. Int. Serv. Santé. Armées.*, 38 :361–363, 1965.
This paper described a form of color lantern and its use in the testing of color vision.

50. NAKAJIMA, A., ICHIKAWA, H., NAKAGAWA, O., MAJIMA, A., & WATANABE, M. Ishihara test in color vision defects. Studies on a statistical method for evaluation of the screening efficiency of several plates. *Amer. J. Ophthal.*, 49 :921–929, 1960.
A set of Ishihara test plates (25 plates from the thirteenth edition) was used for the screening of color vision defects in 3,033 school children aged six to ten years, along with three other plates for tritanopes and anomaloscopic examination for defectives picked up by the screening. Using the number of misread plates as criterion, this set of Ishihara test plates could separate red-green defectives very clearly from normals. Analysis of the distribution of misreadings by statistical methods was presented. The Poisson law can be used for the approximation of the observed distribution, and efficiency in error of screening can be estimated on this basis.

51. PÉCHOUX, R., RESSÉGUIER, J., DEFAYOLLE, M., & RAYMOND, R. Étude d'une batterie d'épreuves pour la détection des anomalies de la vision des couleurs dans les centres de sélection. *Bull. Cent. Étude Rech. Psychotech.*, 6 :171–182, 1957.
This paper compared Beyne's lantern with the orthorater and the Ishihara test. The author recommended that all subjects be examined with the orthorater. Those who obtain scores of eight or more on the scale of ten should be considered normal. Those who score seven or less should undergo the simplified Ishihara test. Those who score exactly eight in the Ishihara test should be considered normal. Those whose score on the Ishihara is less than or equal to seven should be examined by Beyne's lantern.

52. PERDRIEL, G. L'examen de la vision des couleurs en pratique courante. *Annee Ther. Clin. Ophthal.*, 11 :221–237, 1960.
This article surveyed existing color vision tests. It has brief descriptions of the tests but no comparisons were made among them.

53. PERDRIEL, G. Le test de Farnsworth 100 hue. *Ann. Oculist. (Paris)*, 195 :120–130, 1962.
This paper described the principles and uses of the Farnsworth 100 Hue test.

54. PETERS, G. The new Dvorine color perception test. *Optom. Wkly.*, 45 :1801–1803, 1954.
This paper presented some normative data for the second edition of the Dvorine color perception test.

55. PETERS, G. Diagnostic sensitivity of color perception tests. *Optom. Wkly.*, 46 :136–137, 1955.
This paper compared the second edition of the Dvorine color perception test with the American Optical Company color perception test. The author concluded that the second edition of the Dvorine test was a more sensitive diagnostic instrument for the detection of color defects than was the American Optical Company color test (18 plate selection).

56. PETERS, G. A color-blindness test for use in vocational guidance. *Optom. Wkly.*, 48 :1171–1173, 1957.
A quantitative classification table was presented as an aid in the interpretation of the Dvorine color perception test. This provides a classification as to degree of defect, the percentages of color blindness that may be found in the general population for each degree of defect, and an estimate of the vocational limitation resulting from such defects.

57. PICKFORD, R. W. Test for color blindness. *Brit. Med. Bull.*, 9 :82, 1953.
This is a review of the tenth edition of the Ishihara test. The author suggested that the tenth edition was less reliable than the eighth, and that the ninth edition fell between them.

58. PICKFORD, R. W. A practical anomaloscope for testing color vision and color blindness. *Brit. J. Physiol. Opt.*, 14 :2–26, 1957.
A simple filter anomaloscope for testing red-green

and yellow-blue color vision was described. The technique for using the anomaloscope and norms for normal color vision and for color defective vision were provided.

59. PICKFORD, R. W. & LAKOWSKI, R. The Pickford-Nicolson anomaloscope for testing and measuring colour sensitivity and colour blindness, and other tests and experiments. *Brit. J. Physiol. Opt.*, 17:131–150, 1960.
An anomaloscope based on a simple colorimeter was described that permits red-green and blue-yellow tests.

60. RAND, G. & RITTLER, M. C. An evaluation of the AO H-R-R pseudo-isochromatic plates. A test for detecting, classifying, and estimating the degree of defective color vision. *A.M.A. Arch. Ophthal.*, 56:736–742, 1956.
The paper evaluated the AO H-R-R pseudo-isochromatic plates and compared them with the prototype H-R-R tests.

61. RAUTIAN, G. N. A new anomaloscope. (in Russian) *Biofizika*, 2:734–742, 1957.
This paper described an anomaloscope that permits three tests of color discrimination in addition to the usual Rayleigh anomaloscope test.

62. RIU, R. Le problème de la perception des signaux colorés et du Daltonisme dans la marine. *Rev. Med. Nav.*, 11:7–35, 1956.
This paper presented the design for the Beyne chromoptemetric lantern. This lantern was designed to present lights equivalent to the color signal lights used in aviation, in the navy, or on railroads. Stimuli are variable in brightness, size, and duration. The lantern was compared with the lantern currently in use in the French Navy.

63. ROTH, A. Le test 28 hue selon Farnsworth. *Bull. Soc. Ophthal. Fr.*, 66:231–238, 1966.
This paper described a test using 28 hues taken from the Farnsworth-Munsell 100 Hue test and presented together as in the Farnsworth Dichotomous (Panel D–15) test. It was claimed that the test can isolate protans, deutans, tritans, tetartans, and achromats with scotopic vision.

64. SAWA, J. Note on the color vision tests with pseudo-isochromatic test plates. (in Japanese) *Jap. J. Clin. Ophthal.*, 18:1207–1211, 1964.
This paper described several pseudo-isochromatic test plates and assessed their individual characteristics. The author concluded that the Ishihara was the most reliable for screening subjects. He reported that other charts, especially the H-R-R and the TMC, gave equivocal results in borderline cases. For classifying color deficient subjects, the author found the H-R-R charts superior to the TMC charts. He found none of the available charts satisfactory for the determination of the severity of the color deficiency.

65. SCHMIDT, I. Comparative evaluation of the New London Navy lantern for testing color perception. Project No. 21–29–009. August, 1951. USAF Sch. Aviat. Med., Randolph Field, Texas.
The New London Navy lantern test for color perception was evaluated. The author recommended that subjects be adapted to room illumination for one-half hour before testing and that the number of retests in any one series be fixed.

66. SCHMIDT, I. Some problems related to testing color vision with the Nagel anomaloscope. *J. Opt. Soc. Amer.*, 45:514–522, 1955.
Topics dealt with include: elimination of training effects and determination of the total number of readings required to detect minimum practical differences between individuals; application and frequency distribution of the anomalous quotient; determination of the normal matching range of mixtures and of comparison yellow; and test-retest reliability of the Nagel anomaloscope.

67. SEEFELT, E. R. An evaluation of the validity and reliability of the AOC 15-plate pseudo-isochromatic test in routine testing. *Amer. J. Optom.*, 41:371–381, 1964.
This paper compared the classification of 409 airmen by the AOC 15-plate test with the classification determined by the anomaloscope. The author concluded that the AOC 15-plate test lacked the necessary validity and reliability to make it possible to obtain proper color vision classification by a single administration of the test under mass testing conditions.

68. SEEFELT, E. R. A comparison of the AOC and the Dvorine pseudo-isochromatic tests in color vision testing. *Amer. J. Optom.*, 42:250–255, 1965.
Color vision classifications of 210 airmen were determined by the AOC and the Dvorine tests as well as by the anomaloscope. Test results indicated the Dvorine test was a superior dichotomous test when compared to the AOC test. The contention that the Dvorine test might be used quantitatively did not seem to be supported by the data.

69. SEKI, R., OHTA, Y., TAJIRI, K., KURATA, K., & FUKUDA, T. The results on color vision tests by the panel D–15. (in Japanese) *Acta Soc. Ophthal. Jap.*, 68:1312–1323, 1964.
This paper reported the study of color vision testing using the panel D–15 on normal subjects, congenital and acquired color deficient subjects. Many congenital deuteranomalous and protanomalous subjects passed the test with no errors. Many acquired color deficient subjects were classified tritan.

70. SLOAN, L. L. Evaluation of the Tokyo Medical College color vision test. *Amer. J. Ophthal.*, 52:650–659, 1961.
The Tokyo Medical College color vision test was described and evaluated by comparisons with the Ishihara test, twelfth edition, and a special group of 16 plates. The TMC test agreed, with one exception, with a classification determined by the 31 plates of the two other tests.

71. SLOAN, L. L. & HABEL, A. Tests for color deficiency based on the pseudo-isochromatic principle. A comparative study of several new tests. *A.M.A. Arch. Ophthal.*, 55:229–239, 1956.
This paper compared the Ishihara, Dvorine, and AO H-R-R tests. All tests detected the great ma-

jority of those with deficient color perception for red and green. Each of these tests, however, occasionally failed to detect mild degrees of red-green deficiency. Misclassification of normal subjects as color deficient was more likely to occur with the AO H-R-R screening plates than with either the Ishihara or the Dvorine screening tests.

72. Stams, A. Controversial results in examinations of the colour sense by employment of customary methods. *Klin. Mbl. Augenheilk*, 147:261–264, 1965.
When color testing was done with the Stilling-Velhagen tables, the florcontrast and the anomaloscope controversial results were obtained. Relevant factors were mentioned.

73. Topley, H. Sight testing for the merchant navy. *Brit. J. Physiol. Opt.*, 16:36–46, 1959.
This paper described a lantern test for color vision and its use in the screening of applicants for seaman.

74. Tsutsumi, S. A new color vision test. (in Japanese) *J. Clin. Ophthal.*, 18:605–610, 1964.
An attempt was made to convert normal color vision into artificial color defective states corresponding to protanomaly, deuteranomaly, and tritanomaly by wearing cyan, magenta, or yellow filters respectively. The ability for color discrimination was tested under these states; it was found that the chromatic discrimination pattern for red-purple, purple, and blue-purple plates could serve as a guide in determining the kind and degree of color anomaly.

75. Umazune, K., Seki, R., and Obi, S. Trial manufacture of new colour vision test plates. Report 1. (in Japanese) *Acta Soc. Ophthal. Jap.*, 58:732–735, 1954.
This is a report of the test manufacture of the color plates for the Tokyo Medical College color test.

76. Vics, I. I. A clinical evaluation of color testing and color perception. *Amer. J. Optom.*, 43:582–592, 1966.
This paper discussed the use of several color tests for detecting color defects among school children.

77. Walls, G. How good is the H-R-R test for color blindness? *Amer. J. Optom.*, 36:169–193, 1959.
This is a highly critical review of the AO H-R-R test. The author reported that as a normal-abnormal screening device, the test was perhaps as good as the Ishihara and the Dvorine. The author reported that the test made 18% incorrect red-green diagnoses. He concluded that the test is an adequate normal-abnormal screen; he recommended, for serious work, the construction of a simple and inexpensive anomaloscope.

78. Walraven, P. L. & Leebeek, H. J. Recognition of color code by normals and by color defectives at several illumination levels. An evaluation study of the H-R-R plates. *Amer. J. Optom.*, 37:82–92, 1960.
This paper reported the number of errors in color code readings as a function of illumination for subjects grouped according to classification on the H-R-R color plates. For all groups, the number of mistakes decreased as illumination increased. At all illuminations there were more mistakes the more severe the defect according to the H-R-R plates, except at the two highest illuminations where the normals and those with mild defects made equal numbers of mistakes.

79. Walraven, P. L., Leebeek, H. J. & Bouman, M. A. ISCC color aptitude test—The interpretation of some testing results. Report No. WW1956–10. Instituut voor Zintuigfysiologie—R.V.O.-T.N.O. August, 1956.
This paper compared the scores of forty-eight normals and fifty-three color deficient subjects on the Color Aptitude test. The results indicate many color defectives with scores as high as those obtained by normals. The authors, therefore, questioned the value of the CAT as a test for color discriminating ability.

80. Willis, M. P. & Farnsworth, D. Comparative evaluation of anomaloscopes. BuMed Project NM 003 041.2601. Med. Res. Lab. Rpt. No. 190, 18 August, 1952. U.S. Navy Submarine Base, New London, Connecticut.
This study examined six anomaloscopes. It attempted to discover whether one type of instrument was better than another, and to determine the relation of anomaloscope scores to scores from other color vision tests. A combination score was proposed which reduces range and deviation to one figure and which gives an estimate of degree of color deficiency. This scoring method can be used with a comparative scaling technique that can be applied to all anomaloscopes to give comparable scores.

81. Wright, W. D. Diagnostic tests for color vision. *Ann. R. Coll. Surg.*, 20:177–191, 1957.
This is a survey of various tests for color vision. The author concluded that, except for the colorimeter, there was no single test capable of diagnosing all types of defects with certainty. The author suggested that an effective battery of tests should include one of the confusion chart tests, the Nagel anomaloscope, and the Farnsworth-Munsell 100 Hue test.

82. Yamamoto, T. A new qualitative test for color vision. (in Japanese) *J. Clin. Ophthal.*, 16:383–387, 1962.
This paper described a color test plate based on discrimination of chroma. It is used for the classification of protanopic and deuteranopic color defective vision and for quantitatively classifying these defects.

83. Yoshihara, M. Study of objective measurement of color vision (report #2). (in Japanese) *Folia Ophthal. Jap.*, 13:598–610, 1962.
This paper reported measurement for color defect based on the optokinetic nystagmus produced by movement of a specially designed color tablet. Results indicated that the electro-nystagmograms of color-blind subjects were irregular in type and height of waves and in frequency and amplitude of oscillation. With this technique the author claimed success in screening and classifying the degrees of color blindness.

AUTHOR INDEX

Each author's name is followed by the identification number of the reports on which his name appears.

TEST INDEX

Each test is followed by the identification numbers of the reports within which the test is discussed.

PERFORMANCE CHARACTERISTICS OF CONSTANT-FLOW PHASE DILUTION OXYGEN MASK DESIGNS FOR GENERAL AVIATION

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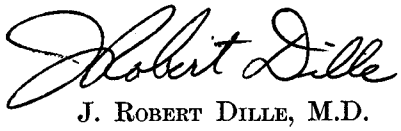

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PERFORMANCE CHARACTERISTICS OF CONSTANT-FLOW PHASE DILUTION OXYGEN MASK DESIGNS FOR GENERAL AVIATION

I. Introduction.

Recent technological advances in general aviation have resulted in the development of aircraft with increased altitude capability and service ceilings. On January 11, 1966, a stock model single-engine Cessna Turbo-System Centurion set a new world altitude record of 39,334 feet for light aircraft.

Oxygen in aviation was first used by French aeronauts and scientists in the famous flight of the Zenith, a balloon ascension which on April 15, 1875, attained an altitude in excess of 8600 meters (28,000 feet)[1]. Oxygen was breathed from goatskin bags through a scented wash bottle and a small rubber tube held in the mouth. Unfortunately, in attempting to conserve their supply of oxygen, these high altitude pioneers became severely hypoxic and lost consciousness. Of the three members aboard the Zenith flight, only one survived. Up until the development of the constant flow BLB mask just prior to World War II, the mouth-held tube or pipestem was still the most common method of administering oxygen in aviation. Not until late in World War II and thereafter were the demand and pressure demand oxygen systems developed.

Constant-flow rebreather type oxygen masks similar in concept to the BLB are most frequently used in general aviation, due to their relatively low cost and oxygen economy afforded at lower altitudes. One basic disadvantage of constant flow oxygen masks is their inherent inability automatically to adjust to the requirements of the user as influenced by his emotional or physical activity.

Dilution of oxygen in constant flow masks is generally afforded by orifices or dilution ports of a predetermined size which allow a varying amount of air to enter the mask, depending upon the oxygen flowing to the mask and the respiratory activity of the individual wearing the mask. Portions of the exhaled gas, higher in oxygen concentration than air, are returned to the rebreather reservoir bag for re-use. Other portions are vented out through the dilution ports, maintaining carbon dioxide at an acceptable level. At higher altitudes, it becomes increasingly difficult to control and assure high concentrations of oxygen with these types of constant flow or economizer masks unless the mask is flooded with oxygen at uneconomical flow rates. With the advent in 1955 of requirements for short duration emergency protection of commercial jet passengers to altitudes of 40,000 feet following the loss of cabin pressurization, the continuous flow phase dilution mask was developed.[2,3,4] This oxygen mask, which conforms to the National Aerospace Standard NAS 1179, does not employ rebreathing and is so designed that 100% oxygen is delivered to the lungs at the beginning of inspiration.[5] This is most advantageous since toward the end of inspiration air is admitted by a valve only after the oxygen in the reservoir is depleted. Air introduced by the valve may penetrate the mask and physiological dead space only. This portion of the tidal volume is the first to be expelled upon initiation of exhalation. Air under these circumstances never reaches the alveoli of the lungs and is therefore physiologically ineffective.

The continuous flow oxygen masks described in this report and designed primarily for general aviation are outgrowths of these developments and incorporate features of rebreathing and phase dilution masks.

II. Methods.

Two prototypes of new experimental rebreathing—phase dilution masks manufactured by the Scott Aviation Corporation were evaluated at altitude on resting and active subjects. These tests were designed to evaluate the physiological adequacy and efficiency of the mask as well as the oxygen system as an entity. For comparative

air, and total oxygen may be derived according to the following calculations:

$$\text{Per Cent Dilution} = \frac{\text{End expired } N_2 \times 100}{N_2 \text{ of air } (79.03)}$$

Oxygen from supply = 100 Per cent of dilution

Oxygen from ambient = Per cent dilution + oxygen from ambient

Total oxygen = oxygen from supply + oxygen from ambient

Calculated inspired oxygen partial pressure = $(P_B - 47)$ x Per cent total oxygen

Where: P_B = Total pressure in mm Hg at ambient altitude.

47 = Pressure, in mm Hg, of saturated water vapor at body temperature.

A Waters ear oximeter was affixed to the antihelix of the ear of each subject in order to obtain blood oxygen saturation under all conditions. A Statham pressure transducer was connected to the system in order to record the line pressure response of the regulator to altitude. Closed circuit television was utilized in order to monitor each subject's condition. Motion picture photography was also used to record the reservoir bag condition and volume. Instrumentation of the subjects is shown in Figure 5.

A bicycle ergometer was modified so that it could be operated by each subject while seated in an aircraft type seat. After the subject was instrumented and ground level baselines recorded, the chamber pressure was decreased to equivalents of 10,000 and 14,000 feet for recording of additional air breathing baselines. Chamber pressure was then increased to an equivalent of 8,000 feet in order to determine the subject's ability to equalize middle ear pressure. After the subject had donned the test mask, the chamber

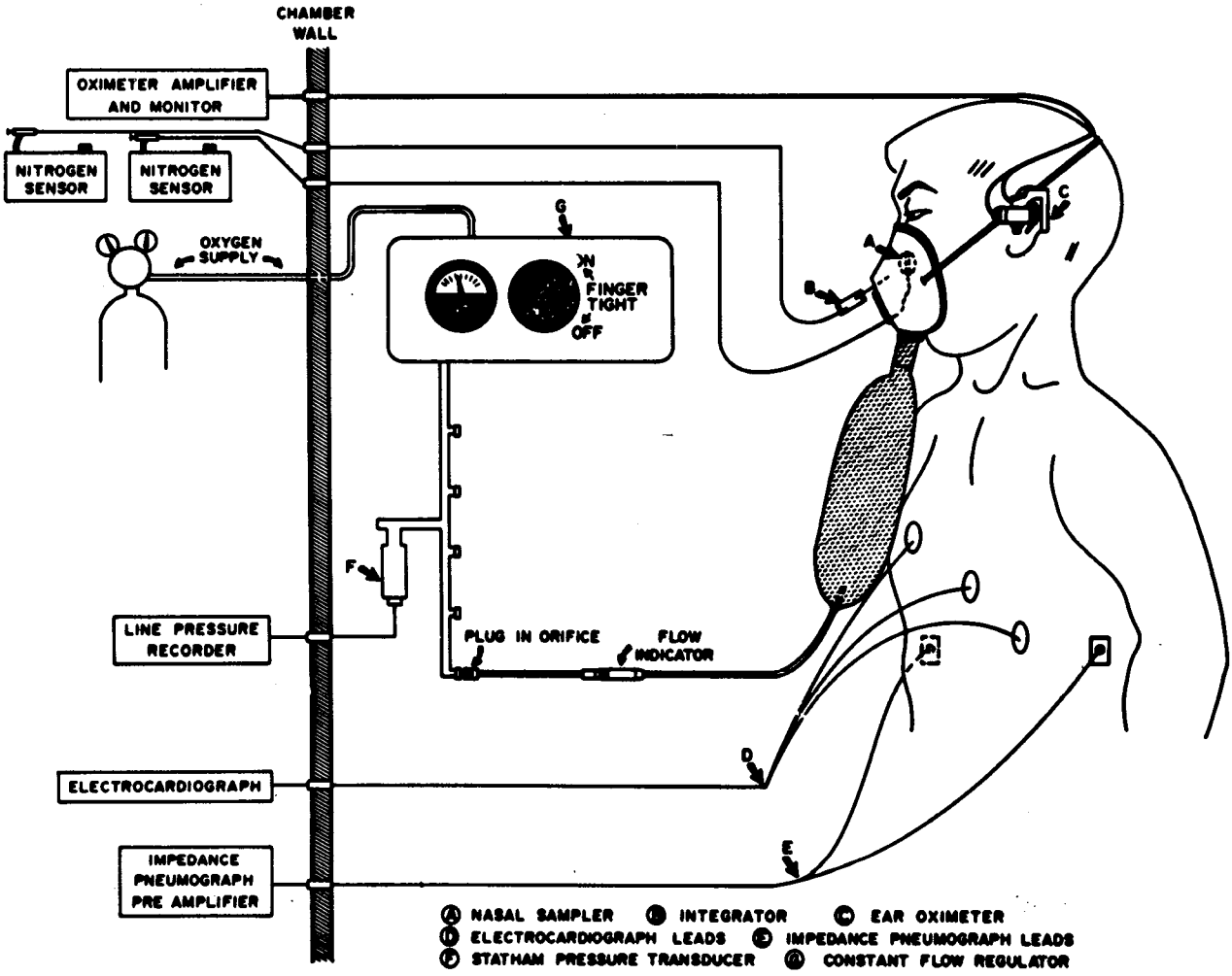

FIGURE 5.—Instrumentation of subjects and diagram of oxygen system.

pressure was decreased to equivalents of 14,000, 18,000, 25,000, 30,000 and 34,000 feet, leveling off at each altitude with the subject engaged in three-minute intervals of rest and light exercise at each altitude (Figure 6).

The bicycle ergometer was operated by each subject at 45 rpm and a workload setting of 45 watts, approximately equivalent to walking at a rate of 3.0–3.4 miles per hour on level terrain.

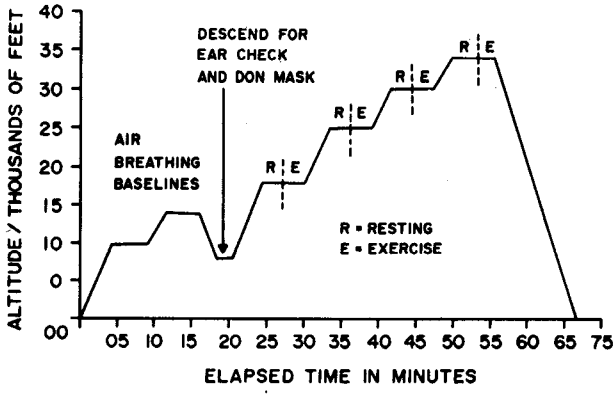

FIGURE 6.—Altitude chamber flight profile.

Oxygen flow provided to the mask is a function of the altitude response of the regulator which produces an increase in line pressure to fixed calibrated plug-in orifices incorporated in the mask hose. As altitude is increased, pressure and resultant flow to the mask are increased.

Oxygen flow, as provided by the regulator and orifices furnished with the mask, was determined by separately exposing the regulator to the same altitudes as the human subjects and recording the flow under standard conditions outside the chamber as shown in Figure 7. The capacity of the regulator to maintain pressure and resultant flow with a single as well as eight orifices connected to the system, was also dedetermined (Figures 8 and 9). Representative oxygen flow rates during the human subject tests with a single outlet in use are shown in the first portions of Tables 4 and 5.

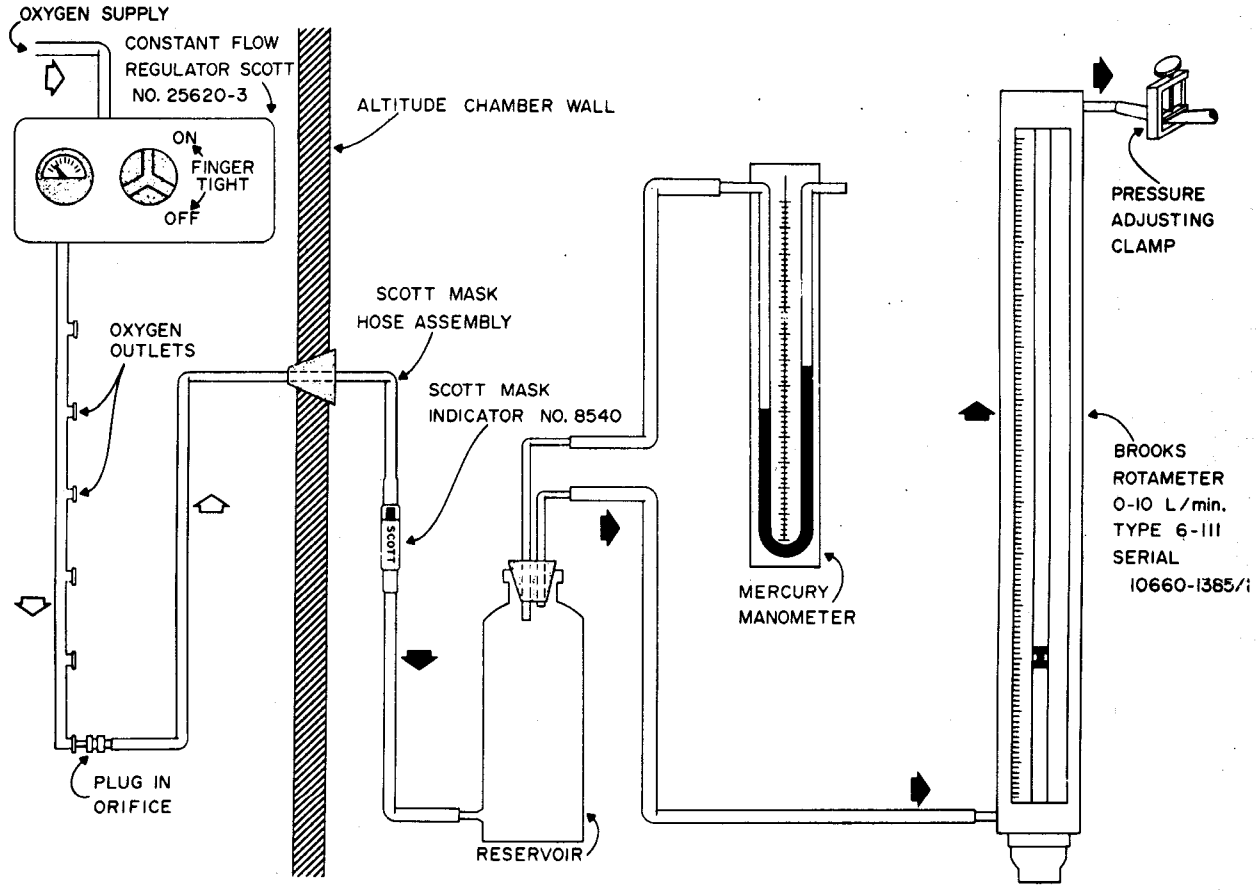

FIGURE 7.—Diagram of test method for determining flow characteristics of the automatic constant flow regulator and orifices. Regulator and outlets installed inside chamber and exposed to altitude.

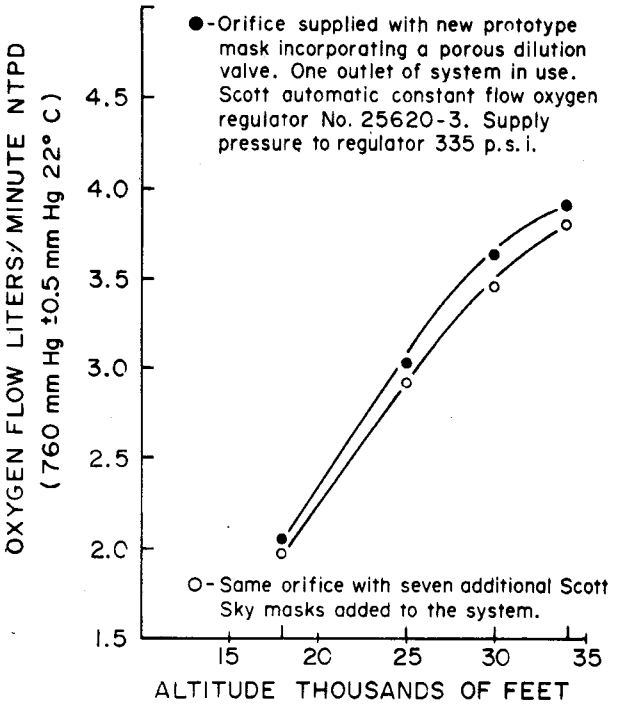

FIGURE 8.—Oxygen flow as determined by regulator and orifice as supplied with mask described above. Plot of data from Table IV.

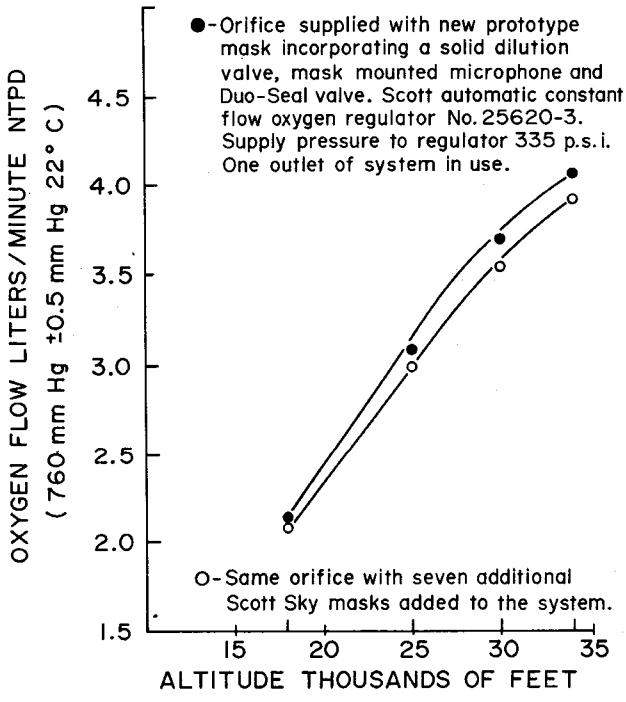

FIGURE 9.—Oxygen flow as determined by regulator and orifice as supplied with mask described above. Plot of data from Table V.

III. Results.

Oxygen flow as provided by the regulator was sufficient to maintain the subjects' indicated blood oxygen saturations well above their 10,000 and 14,000 feet baselines and equalled or exceeded their ground level (1,273 feet) baselines (Tables 1, 2 and 3). It should be emphasized that the masks were carefully fitted to the subjects to provide a good seal to the face. Additionally, all subjects were young, healthy males 24–38 years of age with previous high altitude experience.

From the standpoint of relative efficiency, calculated inspired oxygen partial pressures indicate that the phase dilution masks which incorporated either a porous dilution port or a dilution valve were comparable (Figures 10 and 11). The open dilution port mask produced lower tracheal oxygen partial pressures at all altitudes during rest and exercise. The effect of one minute of reading aloud was evaluated at 30,000 and 34,000 feet with this mask. This activity, due to high peak inspiratory flow rates and mask displacement, reduced the tracheal partial pressure to a greater extent than did exercise (Figure 11). However, since reading was limited to one minute, the reduced tracheal partial pressures did not appear to produce a significant effect upon the blood oxygen saturation.

The flows as provided by orifices furnished with the porous dilution port mask and solid dilution valve may be compared in Tables 4 and 5. Although the orifice furnished with the prototype mask incorporating a solid flutter type dilution valve provided slightly higher flow rates, this appears to be a function of manufacturing tolerance.

IV. Discussion.

The Scott Oxymatic automatic constant flow regulator when equipped with the proper orifices and efficient masks is designed automatically to provide sea level equivalents of oxygen flow to an altitude of 30,000 feet. Physiological evaluation of subjects at altitude indicated that the system, when utilized with new prototype rebreathing masks, maintained the subject's blood oxygen saturation and inspired tracheal oxygen partial pressure equal to or in excess of the ground level equivalent. Masks of this type provided higher and more consistent tracheal

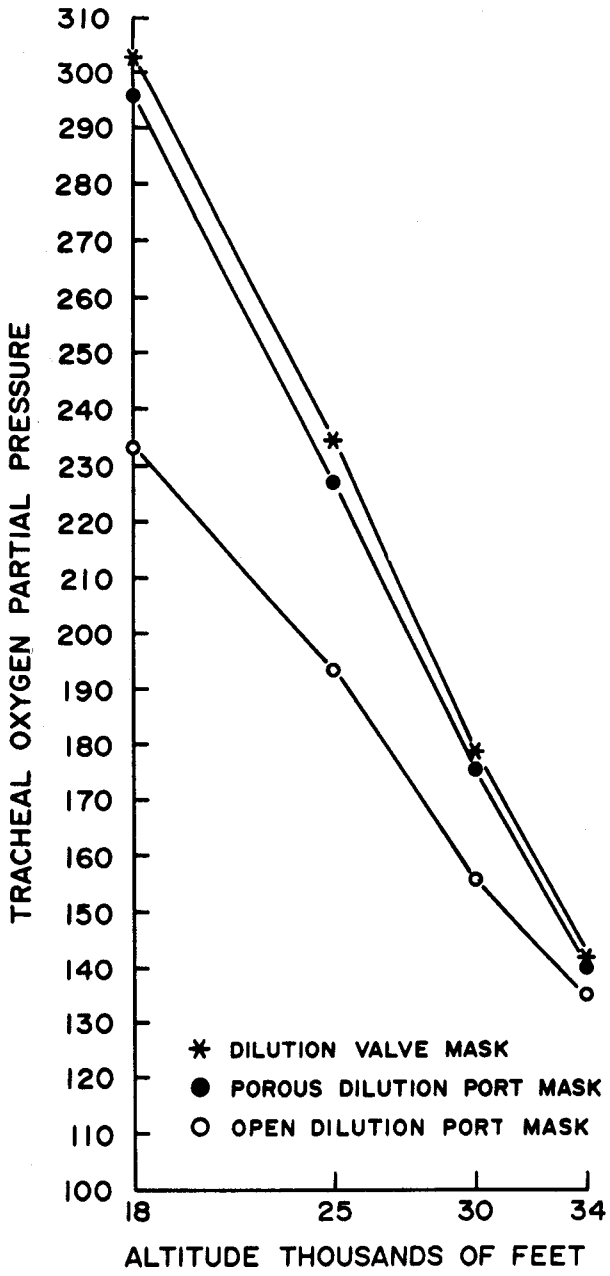

FIGURE 10.—Comparison of calculated resting tracheal oxygen partial pressures. Summary of data from Tables 1, 2 and 3.

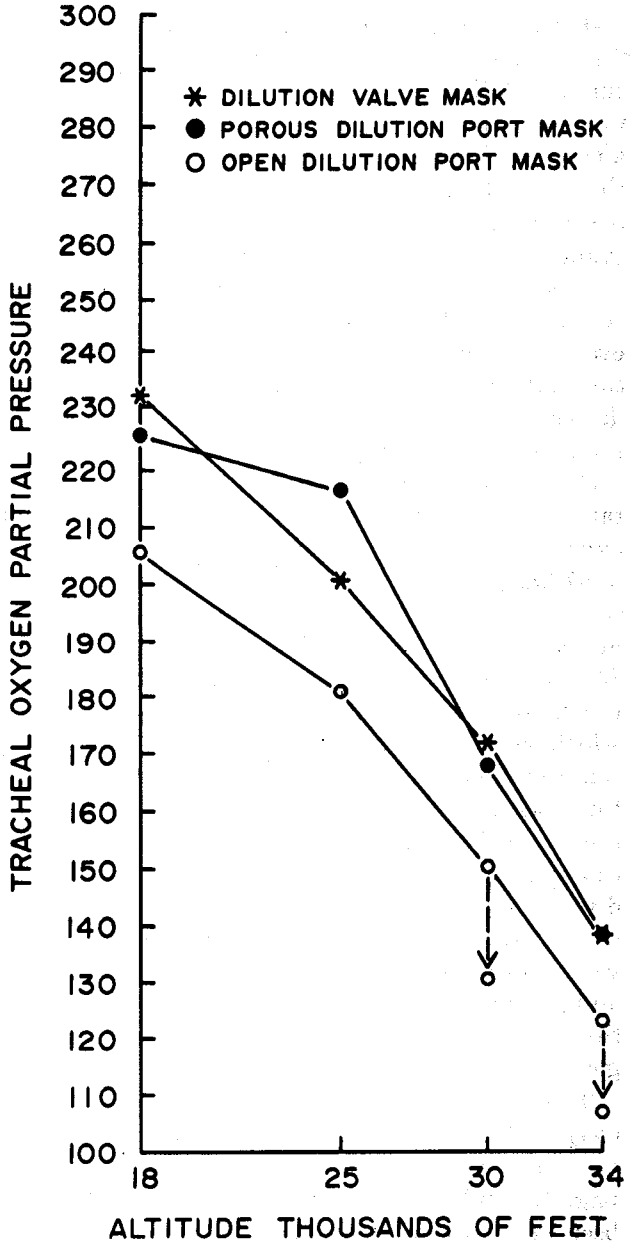

FIGURE 11.—Comparison of calculated tracheal oxygen partical pressures during exercise. Broken lines and arrows at 30,000 and 34,000 feet indicate magnitude of depression of tracheal oxygen partial pressure induced by high inspiratory flows characteristic of speech (reading). Summary of data from Tables 1, 2 and 3.

oxygen partial pressures than the similar mask equipped with open dilution ports. Had oxygen flows been lower it is anticipated that this difference would be exaggerated. In addition, if the tracheal oxygen partial pressure is reduced to a point approximating 90 to 100 mm Hg, hypoxic drive of respiration may ensue. In this case, a vicious cycle may develop when using constant flow diluter masks in that the mask wearer may reflexly hyperventilate. The increased ventilation results in more ambient air being drawn into the mask, diluting the oxygen, which in turn increases hypoxia and hyperventilation, continuing reduction of mask efficiency and resulting in an increased deterioration of the physiological condition of the mask wearer.

Hypoxic hyperventilation does not produce this same effect when air is breathed at, for example, 14,000 feet, since the inspired partial pressure remains constant. Similarly, at higher altitudes when demand oxygen equipment is used, the ratio of oxygen to air is accomplished by the demand regulator which is so designed and programmed that this ratio remains constant at a specific altitude regardless of the ventilation and respiratory activity of the mask wearer. However, with constant flow systems and masks, dilution ratios at a specific flow rate are largely determined by introduction of ambient air into the mask. Mask design, phase of the respiratory cycle in which dilution occurs, inspiratory flow rates and volume are the determiners of the oxygen/air ratios at a specific oxygen flow.

Although it was not measured, CO_2 accumulation in the rebreather bag did not appear to present a problem with the prototype masks. In previous mask evaluations it was found that the use of rapid response infra-red CO_2 analyzers which require relatively high sample flow rates compromised the determinations of end expiratory gases. Since the minimum required sample flow of 250 cc per minute represented a significant portion of the inspired gas, it could not be discarded but was returned by a closed circuit to the rebreather bag. However, as the sample was returned to the rebreather bag continuously and not in phase with the respiratory activity of the subject, measurement of other expiratory gases was rendered erratic and erroneous.

The respiratory center is very sensitive to CO_2 and it is anticipated that had significant quantities of CO_2 accumulated in the rebreather bag, the subject's respiratory rate would have been increased out of proportion to that expected for the degree of activity in which the subject was engaged. These data do not indicate this response.

It must be realized that these evaluations were carried out at altitude on young, healthy male subjects under favorable conditions with the masks correctly donned and providing a good fit to the face. Under actual conditions of use, care and caution may not be extended to this degree.

V. Summary and Conclusions.

1. Oxygen flow as provided by the Scott Oxymatic automatic constant flow regulator, when used in conjunction with either of the prototype phase dilution—rebreather test masks and proper orifices, was sufficient to maintain the subjects' indicated blood oxygen saturations equal to or in excess of their ground level (1,273 feet) baselines.

2. With the same oxygen flow rates the prototype porous and dilution valve masks provided higher tracheal oxygen partial pressures than the open port masks at all altitudes tested during rest and exercise.

3. The slight reduction of flow rates as provided by the Scott Oxymatic regulator when eight orifices were plugged into the system as opposed to one orifice is not considered to compromise significantly the performance of the system.

4. Heart and respiratory rates of all subjects indicate normal response, with the maximum generally occurring during exercise and hypoxia induced during establishment of air breathing baselines at 14,000 feet.

5. At the same flow rates a greater amount of oxygen was derived from the supply and less lost by the prototype phase-dilution rebreather masks as compared to the open port type mask.

TABLE 4. Oxygen flow furnished by the Scott automatic constant flow regulator No. 25620–3 as measured at Normal Temperature Pressure Dry (NTPD) 22°C, 760 ± 0.5 mm Hg, using the orifice supplied with the new prototype version of the Scott Sky Mask. This mask incorporates a porous dilution valve. Automatic flow by increased pressure was activated by exposing the regulator to the indicated altitudes and measured as shown in Figure Seven. One outlet was used and the supply pressure maintained at 335 psi.

Altitude (Feet)	*Barometric Pressure mm. Hg.*	*Line Pressure p. s. i.*	*Flow L/Min NTPD*	*Calculated Flow L/Min BTPS*
0	760	40	0.00	
8,000	565	42	Less than 1.00	
18,000	380	58	2.05	4.93
25,000	282	71	3.02	10.28
30,000	226	74	3.63	16.23
34,000	188	78	3.95	22.44
Same orifice and conditions as above, but with seven additional Scott Sky Masks plugged into the system.				
0	760	41	0.00	
8,000	565	42	Less than 1.00	
18,000	380	58	1.98	4.77
25,000	282	70	2.92	9.94
30,000	226	74	3.46	15.47
34,000	188	78	3.82	21.70

TABLE 5. Oxygen flow furnished by the Scott automatic constant flow regulator No. 25620–3 as measured at Normal Temperature Pressure Dry (NTPD) 22°, 760 ± 0.5 mm Hg, using the orifice supplied with the prototype Scott mask. This mask incorporates a mask-mounted microphone and a phase dilution valve. Automatic flow by increased pressure was activated by exposing the regulator to the indicated altitude and measured as shown in Figure Seven. One outlet used and the supply pressure maintained at 335 psi.

Altitude (Feet)	*Barometric Pressure mm. Hg.*	*Line Pressure p. s. i.*	*Flow L/Min NTPD*	*Calculated Flow L/Min BTPS*
0	760	41		
8,000	565	44	Less than 1.00	
18,000	380	58	2.15	5.17
25,000	282	70	3.09	10.51
30,000	226	75	3.67	16.41
34,000	188	79	4.05	23.01
Same orifice and conditions as above, but with seven additional Scott Sky Masks plugged into the system.				
0	760	40		
8,000	565	43	Less than 1.00	
18,000	380	58	2.07	4.98
25,000	282	71	3.00	10.21
30,000	226	75	3.54	15.83
34,000	188	79	3.92	22.27

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