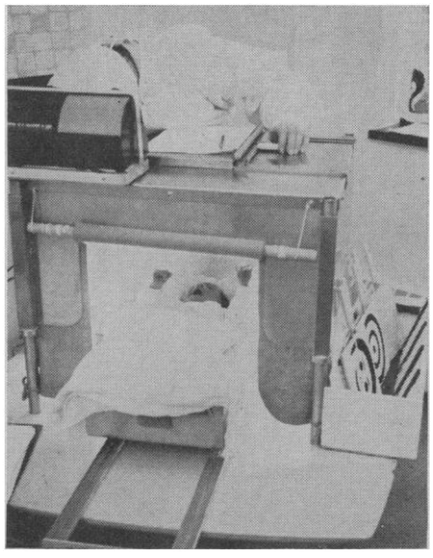

Fig. 1. Infant "looking chamber" for testing visual responsiveness to targets exposed under controlled-stimulus conditions. The patterned objects are visible in a box on the table, each with a handle for sliding it in the chamber. Observer is looking on one side of the target through the peephole, which is hidden by the timer.

containing the patterns or colors were placed in a flat holder which slid horizontally into a slightly recessed portion of the chamber ceiling to expose the pattern or color to the infant through a circular hole in the holder. The chamber and underside of the holder were lined with blue felt to provide a contrasting background for the stimuli, and to diffuse the illumination (between 10 and 15 ft-ca) from lights on either side of the infant's head. The subject was in a small hammock crib with head facing up directly under the targets, 1 foot away.

The results in Table 1 show about twice as much visual attention to patterns as to plainly colored surfaces. Differences in response to the six stimulus objects are significant for the infants both under and over 2 days of age; results from these groups do not differ reliably from each other, and are similar to earlier results from much older infants. The selectivity of the visual responses is brought out still more strikingly by tabulating the longest-fixated target for each newborn infant: 11 for face, 5 for concentric circles, 2 for newsprint, and 0 for white, yellow, and red. For comparison, the first choices of infants 2 to 6 months were distributed as follows: 16, 4, 5, 0, 0, 0.

Three infants under 24 hours could be tested sufficiently to indicate the individual consistency of response. Two of these showed a significant (.005 and .05) difference among the targets in successive sets of exposures, one looking longest at the face pattern in 7 of 8 exposures, the other looking longest at the "bull's-eye" in 3 of 6 exposures. The third infant 10 hours after birth looked longest at the face in 3 of 8 exposures.

It is clear that the selective visual responses were related to pattern rather than hue or reflectance, although the latter two variables are often thought to be primary visual stimuli. Specification of the prepotent configurational variables is unwarranted at this time. The results do not imply "instinctive recognition" of a face or other unique significance of this pattern; it is likely there are other patterns which would elicit equal or greater attention (*5*). Longer fixation of the face suggests only that a pattern with certain similarities to social objects also has stimulus characteristics with considerable intrinsic interest or stimulating value; whatever the mechanism underlying this interest, it should facilitate the development of social responsiveness, since what is responded to must first be attended to.

Substantiation for the visual selection of patterned over unpatterned objects is given in an independent study of newborn infants in which more visual attention was given to a colored card with a simple figure, when held close to the infant, than to a plain card of either color (*6*).

The results of Table 1 demonstrate that pattern vision can be tested in newborn infants by recording differential visual attention; these and other results call for a revision of traditional views that the visual world of the infant is initially formless or chaotic and that we must learn to see configurations (*7*).

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4. High reliability of a similar technique, using the same criterion of fixation, was shown with older infants (*1*). Since eye movements are less coordinated and fixations less clear-cut in newborn infants, a further check of the response measurement is desirable; I plan to do this by photographic recordings.
5. I chose the targets for their expected attention value for the older infants of the earlier study; this may be different for newborn subjects: response to the newsprint may be decreased by less acute vision (although some patterning would be visible without resolution of individual letters); "bull's-eye" elicited strong differential attention only over 2 months of age in another study (*3*); and blue is preferred to red and yellow by newborns (*5*). The face pattern might for these reasons have a relative advantage for newborns.
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7. Supported by research grant M-5284 from the National Institute of Mental Health. I am indebted to Booth Memorial Hospital for making the subjects available; to Major Purser, Caroline Holcombe, R.N., Dr. R. C. Lohrey, and other staff members for their cooperation; and to Isabel Fredericson for invaluable assistance.

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Scavenger Probe Sampling: A Method for Studying Gaseous Free Radicals

Abstract. *Scavenger probe sampling for determining the concentration of certain gaseous free radicals and atoms has been used to study flames and electric discharges. Combining microprobe sampling and chemical scavenging with mass spectral analysis, this technique offers high spatial resolution, absolute concentration determination, and high temperature applicability. The reactions of hydrogen atoms with chlorinated hydrocarbons and oxygen atoms with nitrogen dioxide were used for scavenging. The results were reproducible and proportional to concentration. In an electric discharge oxygen atom concentrations agreed with gas phase titration determinations. The gas phase titration measures atom flux rather than concentration, and differences as high as 20 percent were observed. A method for deriving concentration from flux measurements is discussed.*

Many important chemical reactions involve atoms and free radicals (*1*). These species have only transient existence under normal laboratory conditions and chemical kinetic studies involving them must be undertaken in difficult environments such as flames and electric discharges. A knowledge of the concentrations of these transient species is necessary for such work, but the analytical problem has until recent years been prohibitively difficult. The problem has usually been avoided by the application of the steady-state approximation which allows radical concentration to be expressed in terms of the measurable stable species. In many

important systems such as flames, however, there is some question as to the validity of this approximation (*2*) which assumes

$$d X \text{ (radical)}/dt \approx 0 \qquad (1)$$

In this equation X is mole fraction and t is time. In a flame this is equivalent to assuming thermal equilibrium at every point. Therefore, experimental methods for determining radical and atom concentrations in such systems are desirable. One such experimental method for making studies in gaseous systems is presented here. Scavenger probe sampling combines the techniques of microprobe sampling (*3*) with chemical scavenging (*1*, vol. 1, pp. 54–55) and continuous-flow analysis with a Consolidated Electrodynamics model 21-620 mass spectrometer. Microprobes have been used in the analysis of the structure of premixed flames. A typical probe consists of an uncooled quartz tube ending in a taper of approximately 30° with an orifice of some 10 to 50 microns in diameter. It is operated with a high pressure drop (10 : 1) under continuum-flow conditions. The sample is passed into a mass spectrometer. (Other analytical instruments could have been used.) In such a probe the sample is rapidly (10^{-5} sec) expanded adiabatically, and the resulting drop in pressure and temperature slows the reactions so that the composition is "frozen" very close to the inlet conditions. This quenching or freezing of the composition of a reacting mixture by rapid adiabatic expansion is a well-known phenomenon in nozzle flow (*4*) and allows a meaningful sample to be taken from a fast-reaction system such as a flame. Chemical scavenging is a technique for studying radical concentrations in photolysis and other reactions (*5*). In the usual application a species is added to the system which undergoes a quantitative reaction with the radical to yield a stable product which is then analyzed. The radical concentration is inferred from its known relation to the product species. This technique as previously used is only satisfactory where the addition of the scavenger species does not disturb the system being studied.

The scavenger probe extends the range of microprobe sampling studies to the determination of radical concentrations and avoids the limitations of scavenger studies by isolating the scavenging reactions from the system being studied. The advantages of the technique are (i) good spatial resolution is possible, (ii) concentrations are determined in absolute rather than relative terms, and (iii) it can be used in high-temperature systems. For quantitative studies the decompression, mixing, and scavenger reaction rates must be rapid compared with any other reactions which the radical can undergo, and the scavenger reaction must produce a unique product.

For hydrogen atom studies chlorinated diffusion pump oil (Convachlor 12) was used as the scavenger. The reaction was:

$$H + C_nCl_{2n+2} \rightarrow HCl + C_nCl_{2n+1}$$

and the analyzed species was HCl. A small glass diffusion pump served both as pump and scavenger introducer. The probe was an uncooled quartz tube 20 mm in diameter and 50 mm long, with a 30° tapered tip ending in a 50-μ orifice (Fig. 1). The high pumping speed reduced the residence time in the probe before mixing with the scavenger gas in the diffusion-pump jet. The observed concentration profiles were reproducible and appeared to be pro-

Table 1. Effect of excess NO_2 on measured oxygen atom concentration* (10^{-2} mole fraction).

Excess X_{NO_2}	X_O
0.56	1.29
0.49	1.32
0.30	1.23
0.15	1.23
0.10	1.17

*Methane-oxygen flame of Fig. 2; $Z \approx 1.6$ cm.

Table 2. Effect of sampling pressure level on measured oxygen atom concentration*.

Pressure (10^{-3} mm-Hg)	Mole fraction ($10^{-3} X_O$)
36	6.4
42	6.9
72	6.8
100	7.3
120	7.4
165	7.2

*Methane-oxygen flame of Fig. 2; $Z \approx 2.0$ cm.

Table 3. Comparison between scavenger sampling and gas phase titration on O-atom concentration in a discharge tube.

Velocity (cm/sec)	10^{-2} Fractional molar flux (titration)	Concentration (10^{-2} mole fraction)	
		Titration*	Scavenger
	P = 0.56 mm-Hg		
107	6.30	5.57	5.85
220	2.57	2.38	2.26
	P = 0.67 mm-Hg		
82	6.52	5.68	6.01
	P = 1.24 mm-Hg		
99	2.82	2.69	2.56
99	2.73	2.57	2.68
	P = 1.53 mm-Hg		
80	2.52	2.29	2.34

*Concentration derived from Eq. 1. See Fig. 4.

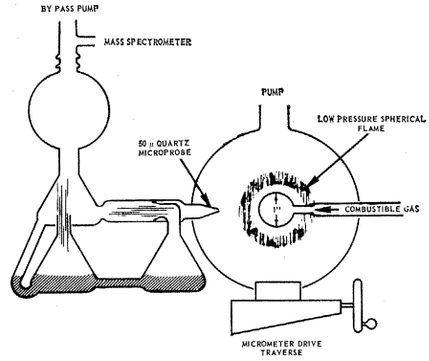

Fig. 1. Scavenger probe apparatus for the study of hydrogen atom concentrations in low-pressure spherical flames.

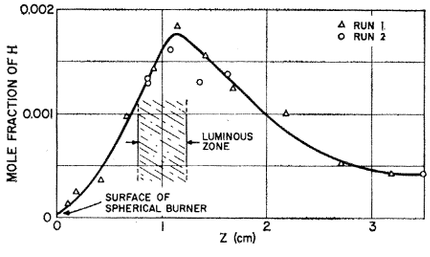

Fig. 2. Hydrogen atom profile of a laminar premixed spherical flame (CH_4, 0.09; O_2, 0.19; Ar, 0.72; P, 3 cm-Hg). Mole fraction is plotted as a function of distance (cm) from the surface of the flame holder.

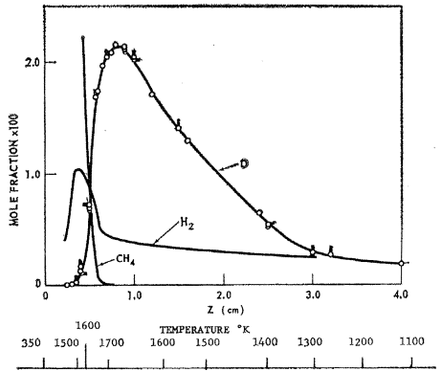

Fig. 3. Oxygen atom concentration in a laminar, premixed, spherical flame (X_{CH_4}, 0.08; X_{O_2}, 0.92; P, 3.5 cm-Hg). The mole fraction is plotted as a function of distance from the surface of the flame holder and temperature (°K). Results are from runs on three successive days. Solid curves showing the concentration of methane and hydrogen are added for comparison. Temperatures were measured with a 0.001-inch diameter silica-coated thermocouple of Pt and Pt-10 percent Rh.

portional to hydrogen atom concentration (Fig. 2), but only a fraction of the calculated adiabatic equilibrium concentration was observed. It was concluded that the efficiency of this system was low. This may be due to recombination on the hot walls of the uncooled quartz probe since it has been shown (*5*) that the recombination coefficients of atoms on silica surfaces go up sharply with temperature.

The technique has also been applied to the study of oxygen atom concentrations in flames (*6*) (Fig. 3) and electric discharges (Fig. 4) in the fast reaction $O + NO_2 \rightarrow O_2 + NO$ for scavenging and detecting NO. For these studies the apparatus of Fig. 1 was modified by the addition of water cooling to the probe and by adding a small tube for injecting NO_2 in the expansion section. It was found desirable to eliminate the diffusion pump which lay between the probe and spectrometer inlet. This reduced the time required to reach equilibrium from 20 minutes to 1 minute. The pumping system (5 lit/sec) of the spectrometer proved to be adequate for these studies. The standard deviation for 20 runs under identical conditions was 2 percent. The results were independent of the NO_2 concentration in a reasonable excess (Table 1) and of sampling pressure above 0.1 mm-Hg (Table 2). To demonstrate that the conversion of NO_2 to NO was due to active species in the sampled flame gases rather than to pyrolysis on the hot probe tip, a silver coil which catalyzed the oxygen atom recombination was introduced between the incoming sample and injected NO_2. This reduced the NO concentration to a negligible value. Upon moving the coil to a point well below the NO_2 inlet, the NO concentration rapidly rose to the original value. The coil developed a black film which appeared to be silver oxide.

To establish the quantitative nature of scavenger sampling it is necessary to compare the results with known concentrations. Quantitative comparison has not been possible in the flame studies because temperatures in the post-reaction zone fall more rapidly than the three body reactions can establish equilibrium (*6*). Therefore, comparison was made by using oxygen atoms generated in a low-pressure microwave discharge and measured by gas-phase titration (*7*) with NO_2.

The comparison between the two techniques was satisfactory (Table 3), as it should be, since identical reactions

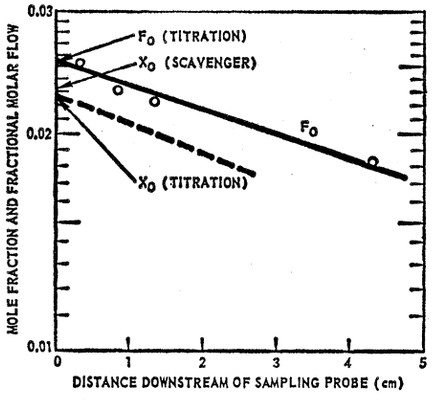

Fig. 4. Concentration and flux of oxygen atoms in the effluent from a microwave discharge in oxygen (see last run, Table 3) flowing down a tube 3 cm in diameter. Mole fraction and fractional molar flux are plotted on a logarithmic scale against distance (cm) downstream of the sampling point. The discharge was located 10 cm upstream of the sampling point and isolated by a choking orifice. At the sampling point the values for the fractional molar flux determined by titration, and the concentration derived from these results by using Eq. 1, are compared with the concentration (mole fraction) determined from the scavenger sampling probe.

are used. This is a demonstration that the sampling process is quantitative. An interesting side point is that in the system which was used the stream velocities were low enough so that a significant difference (10 to 20 percent) existed between fractional molar flux (fraction of the total number of molecules passing through a unit cross section in a unit time) and mole fraction (fraction of molecules per unit volume at a given point) of oxygen atoms (Fig. 4 and Table 3). The difference is due to the effects of molecular diffusion.

The relation between the two variables in a one-dimensional system is given by Eq. 2. The justification of the one-dimensional approximation for such Poiseulle flow systems has been discussed by Walker (*8*).

$$F = X\left(1 - \frac{D}{\bar{v}} \cdot \frac{d \ln X}{dz}\right) \qquad (2)$$

In this equation D is the diffusion coefficient (cm²/sec). For oxygen atoms in oxygen (*9*) at 300°K, $D = 0.3P^{-1}$, where P is pressure in atmospheres, F is the fractional molar flux, v is the velocity (cm/sec) determined from the mass flow, pressure, temperature and tube cross section with a small correction for the probe disturbance, X is the mole fraction, and z is distance downstream of the probe sampling point (cm). F, $\bar{v}$, and X are functions of z.

The concentration gradient was evaluated from the measurements of flux with the initial assumption that $dX/dz \approx dF/dz$. Since the difference between flux and concentration was not large, a single iteration was sufficient.

The titration measures total flux at the point of titration whereas the scavenger probe measures the concentration at the point of sampling. The sampling was in the continuum-flow regime, and sonic velocity in the probe throat is large compared with diffusion velocities. Under these conditions, the composition sample taken by a probe is very closely the concentration at the sampling point (*10*). A second source of difficulty with the titration technique is the effect which the titration may have on the discharge (*11*) through changing the oxygen atom concentration gradient. This was eliminated in this system by isolating the discharge from the main tube by a choking orifice.

The titration was satisfactory except in the immediate vicinity of the probe. Values could be obtained at this point by closing off the sampling probe. A typical set of determinations is shown in Fig. 4.

Recently it has been shown that hydrogen atoms can also be titrated by NO_2 (*12*). Therefore, a correction would be required in systems containing appreciable hydrogen atoms. In the systems discussed here the H atom correction is considered negligible. It should also be noted that methyl radicals have been detected in a methane-oxygen flame according to the scavenger reaction (*13*):

$$CH_3 + I_2 \rightarrow CH_3I + I$$

A water-cooled scavenger probe was used in this experiment (*14*).

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7 February 1963

Acetylcholine and Cholinacetylase Content of Synaptic Vesicles

Abstract. *Acetylcholine, cholinacetylase, and acetylcholinesterase were determined in three subfractions that resulted from the osmotic shock of the "mitochondrial" fraction of the rat brain. Acetylcholine and cholinacetylase were found concentrated in the subfraction that contained mainly synaptic vesicles and some membranes, whereas the larger proportion of the acetylcholinesterase was observed in the subfraction that contained torn nerve endings. These results support the idea that synaptic vesicles are the morphological units of acetylcholine within the synapse.*

Recently a technique was developed in our laboratory (*1*) that permits the separation of synaptic vesicles from isolated nerve endings. This consists in treating with distilled water a freshly prepared "mitochondrial" fraction of the rat brain, in which the presence of numerous intact nerve endings, together with free mitochondria, synaptic membranes, and myelin fragments, had previously been demonstrated (*2*). This procedure results in the swelling and breaking of the nerve endings, with liberation of most of the synaptic vesicles. By centrifugation at 11,500*g* for 20 minutes and further centrifugation of the supernatant at 100,000*g* for 30 minutes, the osmotically disrupted mitochondrial fraction M was separated into three subfractions: (i) M_1 (composed of myelin fragments, swollen mitochondria, and remnants of disrupted nerve endings), containing 31.5 percent of the protein of the total homogenate and practically all the succinodehydrogenase; (ii) M_2 (composed mainly of synaptic vesicles and some membranes), containing 6.3 percent of the protein; and (iii) M_3, a soluble supernatant containing 11.2 percent of the protein (*1*).

In such preparations we have studied the content of acetylcholine, cholinacetylase, and acetylcholinesterase. As shown in Table 1, only about one-third of the acetylcholine and cholinacetylase remains in subfraction M_1 after the osmotic shock; the rest is distributed between M_2 and M_3. Although both acetylcholine and cholinacetylase are mainly localized in M_2, as demonstrated by the relative specific activity (rsa), the acetylcholine occurs in lower concentration. This could be explained by the greater solubilization of acetylcholine into M_3.

The fact that the highest concentration of acetylcholine and cholinacetylase is found in subfraction M_2 is interpreted as a demonstration that the synaptic vesicles are indeed the carriers of this transmitter substance, as had been postulated by De Robertis and Bennett (*3*), and that they contain the enzyme directly involved in its synthesis. The concentration of acetylcholine and cholinacetylase in the synaptic vesicles is better shown by the expression (rsa M_2)/(rsa M_1), the ratio between the concentrations of succinodehydrogenase, acetylcholinesterase, acetylcholine, and cholinacetylase in the two structural compartments of the nerve endings.

After the osmotic shock, most of the synaptic vesicles appeared to have small discontinuities or pores in the membrane which allowed phosphotungstate to penetrate, and 30 percent of the acetylcholine had been liberated into subfraction M_3. In spite of these discontinuities, which probably resulted from the hyposmotic treatment, a large part of the acetylcholine remained bound to the vesicle—a finding which suggested that it might form a complex with the synthesizing enzyme or other components in the vesicular membrane. As shown by Whittaker (*4*), the total amount of acetylcholine can be released only through more drastic procedures involving denaturation of the proteins.

The higher concentration of cholinacetylase in M_2 and its lesser solubilization into M_3 indicate that most of this enzyme is firmly bound to the structure of the vesicular membrane.

Acetylcholinesterase, although preferentially localized in cholinergic nerve endings (*2*), differs in being more finely distributed within the nerve-ending complex. Most of it remains in subfraction M_1, which contains the remnants of disrupted nerve endings. Its high relative specific activity in M_2 could be explained by the low protein content and the presence of synaptic membranes in this subfraction. However, the ratio (rsa M_2)/(rsa M_1) is rather low.

The foregoing facts and some preliminary results of the subfractionation of M_2 suggest that acetylcholinesterase may be localized in the membranes of the ending at the synaptic junction. Another interesting difference in the

Table 1. Protein content and activities of enzymes and other active substances in mitochondrial subfractions M_1, M_2, and M_3 after osmotic shock. The values for the subfractions are means expressed in percentages of the total amount of homogenate recovered and relative specific activity.* Protein was determined with the biuret method of Palladin (*7*); succinodehydrogenase (SDH), with the technique of Slater and Bonner (*8*), involving a change in optical density of a solution of ferricyanide; acetylcholine (ACh), with a modification of the rat-fundus method of Vane (*9*); and cholinacetylase (ChAc), with the method of Hebb and Smallman (*10*), in which acetylating enzyme, choline, acetate, and adenosine triphosphate are used.

Ultrastructure	Determinations (N)	M Absolute values (per gram of fresh tissue)	M_1 (Myelin; mitochondria; ruptured nerve endings) Percentage	M_1 Relative specific activity	M_2 (Synaptic vesicles; membranes) Percentage	M_2 Relative specific activity	M_3 (Soluble fraction) Percentage	M_3 Relative specific activity	(rsa M_2)/(rsa M_1)
Protein	8	33.0 mg	31.5		6.3		11.2		
SDH	5	615.5 U	85.0	2.7	2.3	0.3	0	0	0.1
AchE	3	253.1 U	37.6	1.2	14.6	2.3	0	0	1.9
ACh	4	0.75 μg	21.5	0.7	22.7	3.6	17.0	1.5	5.8
ChAc	4	140.0 U	22.7	.7	35.5	5.6	9.8	0.9	7.8

*For example, the relative specific activity for succinodehydrogenase = (percentage of recovered SDH)/(percentage of recovered protein).