In the Laboratory

An Instrument for Determining the Partial Pressure of Oxygen in a Gas[1]

LINUS PAULING, REUBEN E. WOOD, and J. H. STURDIVANT

The Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena

In response to a request by the National Defense Research Committee, we developed in the Fall of 1940 a simple and effective instrument for determining the partial pressure of oxygen in a gas. The operation of this instrument depends upon the fact that the magnetic susceptibility of oxygen is very much greater than that of any other common gas: at 20° C. and one atmosphere the volume magnetic susceptibility of oxygen is $+142\times10^{-9}$ c.g.s.m.u., whereas that of nitrogen, which is representative of the common diamagnetic gases, is -0.40×10^{-9} c.g.s.m.u. The magnetic susceptibility of a gas is hence determined almost entirely by the partial pressure of the molecular oxygen in the gas;[2] the effect of adding one atmosphere of nitrogen is equal to that of decreasing the partial pressure of oxygen by 0.0028 atmospheres.

The indication of the magnetic susceptibility of the gas in this oxygen meter depends on the behavior of a small test body surrounded by the gas in an inhomogeneous magnetic field. The force on the test body due to the magnetic field is proportional to the difference in volume magnetic susceptibility of the test body and the gas surrounding it. The operation of the instrument is accordingly analogous to the ordinary determination of the density of a liquid by weighing a solid body suspended in the liquid (Archimedes' principle). The test body in the meter is a small dumbbell consisting of two thin-walled glass spheres about 3 mm. in diameter connected by a small glass rod or tube about 4 mm. long; this dumbbell, which weighs about 2 mg., is cemented, together with a small mirror, to a silica fiber about 3 microns in diameter and about 10 mm. long which is stretched across a silica fork. By rotating around the silica fiber, which serves as a torsion balance, the spheres of the dumbbell may move in an inhomogeneous magnetic field. The field is produced by permanent magnets (usually two Alnico V horseshoe magnets weighing about 5 ounces apiece) with suitably shaped soft iron pole pieces. The magnetic susceptibility of the gas in the chamber (with gas volume about 4 ml.) surrounding the test body may be indicated on a scale by a beam of light reflected from the small mirror.

Several hundred of the meters have been manufactured, the first few dozen by the California Institute of Technology, and the others by Dr. Arnold O. Beckman, 11 West State Street, Pasadena 2, California, who took over the production of the meter in the Summer of 1942. The meter is made in several models for various special purposes and to cover various ranges of partial pressure of oxygen. The precision is dependent on the range; it is, for example, about ±1 mm. of mercury for a meter covering the partial pressure range 0 to 180 mm. of mercury. Some recording models of the instrument are available.

Our principal collaborators in the development of the instrument at the California Institute of Technology were David P. Shoemaker, James B. Edson, Harold Herd, Dr. Herbert Sargent, Dr. Charles D. Wagner, and Beckie Bradford. Professor H. Victor Neher gave us valuable advice and instruction in silica-fiber technique.

[1] This work was done in whole under Contract No's. NDCrc–38, NDCrc–200, OEMsr–326, and OEMsr–584 between the California Institute of Technology and the Office of Scientific Research and Development, which assumes no responsibility for the accuracy of the statements contained herein.

[2] The paramagnetic gases, nitric oxide, nitrogen dioxide, and chlorine dioxide, if present, would also contribute largely to the magnetic susceptibility of the gas.

Quantitative Differentiation of Minute Amounts of the Coproporphyrin Isomers (I and III) Based on Fluorescence Behavior[1]

SAMUEL SCHWARTZ, VIOLET E. HAWKINSON, and C. J. WATSON

Department of Medicine, University of Minnesota Hospitals

The coproporphyrin isomers have identical absorption and fluorescence spectra (*1*). Other physical characteristics of differential nature, such as the ester melting point and pH fluorescence curves, require relatively large amounts of crystalline material. The concentration of coproporphyrin in normal and pathological urines is usually in the range of 50–500 γ per 24-hour sample, so that any method which requires crystallization prior to determination of the percentage of isomers in the mixture is unsatisfactory, simply

[1] Aided by grants from the John and Mary R. Markle Foundation, New York City, and the Medical Research Fund of the Graduate School, University of Minnesota.

because of the time, effort, and expense involved in the isolation of crystalline ester. Essentially the same is true in the case of feces. For the purpose of studying the excretion of the coproporphyrin isomers in health and disease, a relatively simple method was needed which could be applied to small amounts of urine and feces. Some time ago a procedure was described which permitted determination of the percentage of isomers in a mixture of very small amounts (*2*). This depended upon a differential elution of the esters from a Tswett column of aluminum oxide. It was found impossible, however, to apply this method to urine with uniform results except where the porphyrin ester had first been crystallized. This was true even though several hundred alternative methods of purification, preliminary to the elution, were tried. Thus, although the method has considerable fundamental interest and value, it is not of practical usefulness.

The purpose of the present communication is to record another and hitherto undescribed difference in physical behavior of the coproporphyrin I and III esters. This consists in a marked difference in rate of precipitation in 30-per cent acetone when the range of concentration of the total coproporphyrin is from 25–70 γ per 100 cc. Since as little as 10 cc. of final solution are required, it is evident that the method is applicable to the resolution of mixtures of from 3 to 7 γ of total coproporphyrin. The phenomenon is characterized by a rapid disappearance of red fluorescence in Wood's light in the case of the type I isomer, while with the type III isomer the disappearance is negligible within 1½ hours. In this range of concentration the solution is colorless, and the precipitation is not visible. It is probable that the phenomenon is actually one of colloidal aggregate formation which then proceeds to actual precipitation. Thus, it has been noted that after standing for 1 hour in a 30-per cent aqueous acetone solution, a considerable amount of coproporphyrin I ester will have collected on the sides of the glass tube. At this time the red fluorescence has disappeared, but if the fluid is poured out of the tube and a small amount of chloroform is then poured in, the chloroform quickly gains an intense red fluorescence in Wood's light, obviously having dissolved porphyrin ester which had been deposited on the glass out of the 30-per cent acetone solution.

The data shown in Fig. 1 reveal the striking difference in behavior of the coproporphyrin esters I and III in 30-per cent aqueous acetone at 4° C. The disappearance of fluorescence is slower at room temperature. It is apparent in Fig. 1 that the rate of decline of the fluorescence intensity is considerably greater as the concentration increases above 30 γ per 100 cc. Thus at 30 minutes the fluorophotometer reading decreased from 150 to 80, with a concentration of 30 γ per 100 cc., while with 60 γ per cent the decrease at 30 minutes was from 260 to 20.

The distinction of the two isomers on this basis is not an absolute one, since there is also a slight diminution of the fluorescence of coproporphyrin III. The difference in this respect is so great, however, that it permits an approximate determination of the amount of each isomer in a mixture. It should be emphasized that the fluorescence of the coproporphyrin III diminishes more rapidly at higher concentrations, so that these are not suitable for application of the method.

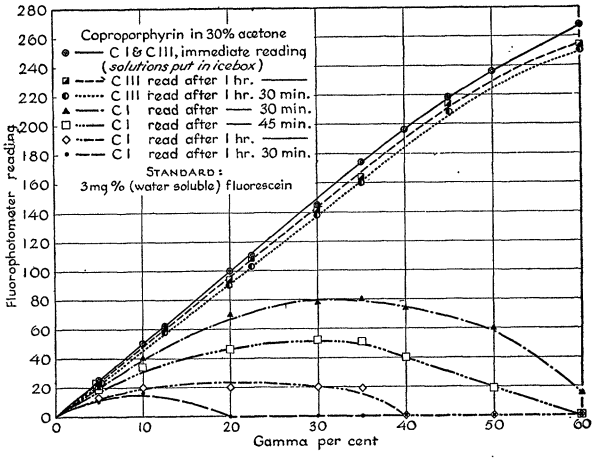

FIG. 1. Fluorescence intensity of the coprophyrin methyl esters (isomer types I and III) in 30-per cent acetone at 4° C. The immediate reading at 0 time was made prior to chilling. The time is constant for all points on each of the curves. The diminution of fluorescence intensity for any given concentration is thus represented by the distance from the 0 curve (immediate reading) to the appropriate curve below.

During the past two years the method has been satisfactorily applied to the coproporphyrin analysis of many 24-hour urine samples, both from normal individuals and in various diseases, 50–250 cc. aliquots being employed for the determination. A considerable preliminary purification is necessary, including ether extraction of the porphyrin from the urine, concentration from the ether by extraction with 5-per cent HCl, methyl esterification, and chromatographic separation of the total coproporphyrin ester on a Tswett column of calcium carbonate, following which the fluorescence intensity in 30-per cent aqueous acetone is measured in a Klett fluorophotometer, using a 3-mg.-per cent aqueous solution of fluorescein as a standard. A reading is taken at once and again after the solution has stood at 4° C. for 1½ hours. The approximate proportions of coproporphyrins I and III in the original mixture are then determined by reference to the data shown in Fig. 1. The validity of the results

for both urine and feces has been confirmed repeatedly by means of isolation of the crystalline ester and determination of its melting point. This, of course, merely establishes the predominating isomer type in the original mixture.. No discrepancies have been encountered between this and the differential precipitation method as described above.

In order to avoid the necessity of correction for the small residual fluorescence of coproporphyrin I when its initial concentration is less than 20 γ per cent, and for the slight diminution in fluorescence intensity of coproporphyrin III at all concentrations employed (see Fig. 1), a curve has been prepared for various mixtures of the isomers, reference to which permits determination of the percentage of each in a given mixture. These data, together with details of the method as applied to urine and feces and the results obtained in various diseases, will be described in separate communications.

References

1. FISCHER, H., and ORTH, H. *Die Chemie des Pyrrols.* (Bd. II, 1. Hälfte.) Leipzig: Akademie Verlagsgesellschaft, 1937.
2. WATSON, C. J., and SCHWARTZ, S. *Proc. Soc. exp. Biol. Med.*, 1940, **44**, 7.

The Use of Ultraviolet Light in Tracing the Course of a Drug Through the Body

CHARLES H. TAFT

Medical Branch, The University of Texas, Galveston
and
The Marine Biological Laboratory, Woods Hole, Massachusetts

It seems possible, if one takes advantage of the fluorescence of various drugs under ultraviolet light, to trace their course through an animal's body. A timetable of the drug's appearance in the different organs might be set up. The greatest concentration of a drug in, and the disappearance of the drug from, a given organ might be judged by the degree of fluorescence of that organ. This is shown to be true in the case of the quinine derivative, quitenine, which is prepared by oxidizing the vinyl group of quinine to the carboxyl group with a permanganate.

Quitenine was chosen for this purpose because of its intense purple fluorescence and because it can be injected in a fairly sizable dose (2 mM/kg.) without killing the animal. The toadfish (*Opsanus tau*) was used as the experimental animal since considerable knowledge of the action of quitenine on this animal is available (*1*). The drug in doses of 2 mM/kg. as quitenine dihydrochloride was injected subcutaneously into the side of the fish behind the dorsal fin. Sloughs developed at the site of injection in a few days. The fish were killed by a blow on the head and opened just before examination. The source of ultraviolet light was a Hanovia Chemical and Manufacturing Company Inspectolite, which gives a light of about 3,660 A.U.[1] All observations were made in a dark room, with the eyes well dark adapted.

In visible light there is not any significant difference in the appearance of the viscera of the uninjected and injected fish except that there is more variation in the color of the gall bladder in the injected fish. Under ultraviolet light the organs of the uninjected fish show no fluorescence with the following exceptions: the gall bladder shows a faint yellowish fluorescence, and in some cases the full urinary bladder shows a strong white fluorescence.

The injected toadfish were killed at daily intervals, until they showed no more fluorescence than the uninjected fish, and examined under ultraviolet light. For the first six days the markings on the skin are much more noticeable in the injected than in the uninjected fish. The injected animal fluoresces, this being especially noticeable in the eyes. For the first two days after injection the cut edges of the skin and muscles show marked purple fluorescence as does the mixture of blood and body fluid that is in the body cavity. The gut shows a strong purple fluorescence for two days and a weaker one the third day. There is no marked fluorescence at the site of injection until a slough develops. The slough gives a strong purple fluorescence until about the seventh day after injection and then gradually weakens, giving a red fluorescence, probably due to some infection. Organs other than the liver, gall bladder, kidney, ureters, and urinary bladder do not fluoresce with the exception of the ovaries, in which at times the eggs, in injected and uninjected fish, give a brilliant golden fluorescence. This is probably connected with their stage of development and is independent of the drug.

These observations show that when quitenine is injected subcutaneously into the toadfish it is at first widely distributed throughout the body and that a large part of it is rapidly concentrated in the liver. The kidney is a very dense organ, and it would require a high concentration before any fluorescence would show. This is attained by the fourth day, although the excretion had begun on the second day, as is shown by the fact that the craniad end of the ureter shows a purple fluorescence. The drug in the liver begins to disappear on the ninth day, that organ being practically free of the drug by the tenth day. The concentration in the kidney becomes low also on the ninth day, and in at least some fish the detectable concentration in the urine disappears on the tenth

[1] The writer is indebted to Dr. George A. Lavin of the Rockefeller Institute for Medical Research for the use of his ultraviolet lamp and for much needed advice.