

by ultraviolet light. They found that a divalent carbon species (methylenes) and a monovalent nitrogen species (nitrenes) represented two classes of triplet state molecules which had the stable characteristics of being in the ground state.

Some ingenious experimental methods were presented for the study of radical reactions and intermediate radical species. A new technique was introduced by J. E. Bennett and A. Thomas ("Shell" Research, England) who used a rotating cryostat for direct measurement of rates of radical-molecule reactions. The rotating cryostat served as a "conveyor belt" on which radicals were first frozen and then bombarded by molecules for specific reactions. The reaction products were then examined by an ESR spectrometer. Another new technique was initiated by P. L. Kolker, T. J. Stone, and W. A. Waters (Oxford University) for the study of transient free radicals involved in oxidation and reduction processes. By appropriately injecting the reactants, they were able to observe intermediate radical species when the reaction products passed through the ESR spectrometer at a very high flow rate. It was possible to establish whether the observed species were the primary or secondary products of a reaction sequence. The information on the identity of transient radicals should throw light on the mechanism of chemical reactions and the nature of electron transfer for oxidation and reduction processes.

C. K. JEN

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Spectrophotofluorometry: Biological Techniques

The initial extramural activity of the new Instituto Di Ricerche Farmacologiche "Mario Negri" in Milan, Italy, was the organization of lectures and demonstrations on spectrophotofluorometric techniques in biology, given by an invited international staff and 110 participants from 19 countries. A NATO grant aided in the financing, and the institute provided the fine facilities of its laboratories and lecture hall now being completed in Milan. The institute, directed by Silvio Garrattini, was founded by a bequest of Mario Negri, a Milanese philanthropist.

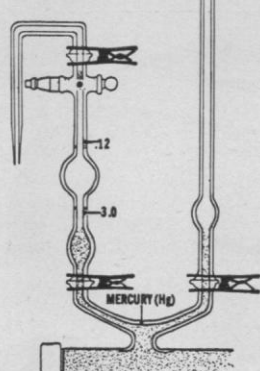
The program was organized as a survey course. Practical experience and

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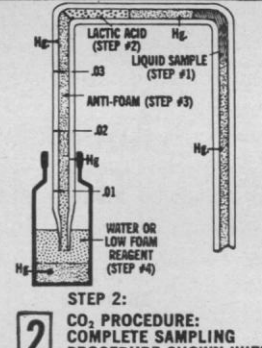
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for Gas Determinations of Ultra-Micro Samples

**SIX
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STEPS**

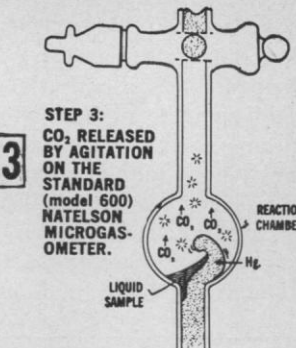


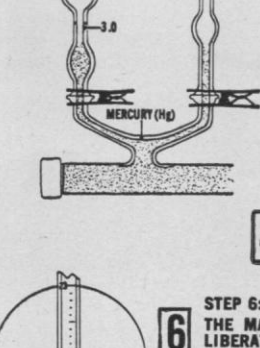
STEP 1:
SYSTEM UNDER
REDUCED PRESSURE
TO REMOVE ALL AIR
PRIOR TO SAMPLING.



STEP 2:
CO₂ PROCEDURE:
COMPLETE SAMPLING
PROCEDURE SHOWN
WITH REAGENTS AND MERCURY
SEALS IN THE PIPETTE.

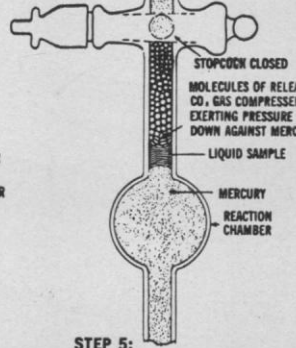
STEP 3:
CO₂ RELEASED
BY AGITATION
ON THE
STANDARD
(model 600)
NATELSON
MICROGAS-
OMETER.

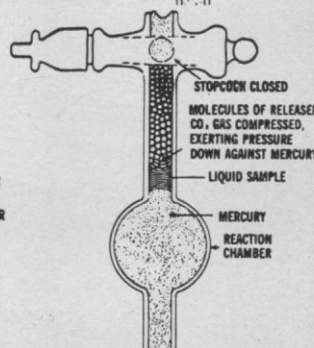




STEP 4:
CO₂ RELEASED BY AGITATION
ON THE MOTORIZED (model
650) NATELSON MICROGAS-
OMETER.

STEP 5:
THE MOLECULES OF RE-
LEASED CO₂ GAS COM-
PRESSED SHOWN EXERTING
PRESSURE DOWNWARD ON
THE MERCURY.

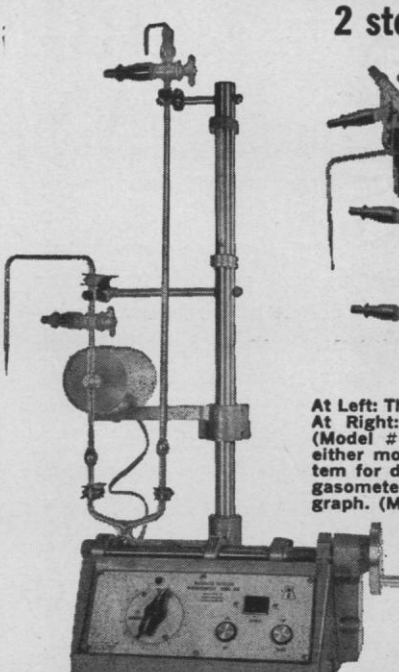




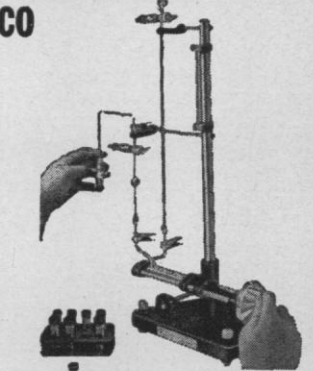
STEP 6:
THE MANOMETRIC PRESSURE (P₁) AS A RESULT OF COMPRESSING THE
LIBERATED CO₂ IN THE REACTION CHAMBER. THROUGH SELECTIVE
ABSORPTION WITH 3N NaOH, P₂ IS OBTAINED.

P₁ - P₂ x Temperature Factor vol. % = CO₂ vol. %

... and you're **1 step from O₂**
2 steps from CO



At Left: The New Natelson Microgasometer (Model #650).



At Right: Standard Manual Natelson Microgasometer (Model #600). Above: The Gas Injector Attachment for either model Natelson Microgasometer. This is the system for direct injection of gases liberated in the Microgasometer. These gases are fed into a Gas Chromatograph. (Model #M-373-25).

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demonstrations were offered on American and European instruments loaned for the occasion. Theoretical and practical considerations determining the design and use of currently available instruments were reviewed and demonstrated by Bowman (National Institutes of Health, Bethesda, Md.) and Hower-ton (American Instrument Company, Silver Spring, Md.). They pointed out the possibilities of increasing sensitivity by utilizing the options offered by the instruments available. The use of mercury xenon sources, specially blazed gratings, and photomultipliers with optimal spectral characteristics, as well as micro cells or phosphorescence attachments, was discussed and demonstrated.

The high sensitivity, rivaling that of bio-assay, the specificity afforded by the activation and excitation spectra, and the fact that radioactive labels and counting are not necessary make the method attractive.

Details of the various commercial instruments illustrate the compromises in spectral resolution and photometric accuracy which are made in the interest of obtaining high sensitivity. The advantages of the use of spectra in identifying sources of blank emission, second-order scatter peaks and Raman lines that overlap the region of emission, and the ability to select working wavelengths that eliminate these were demonstrated in the laboratory sessions. Once these

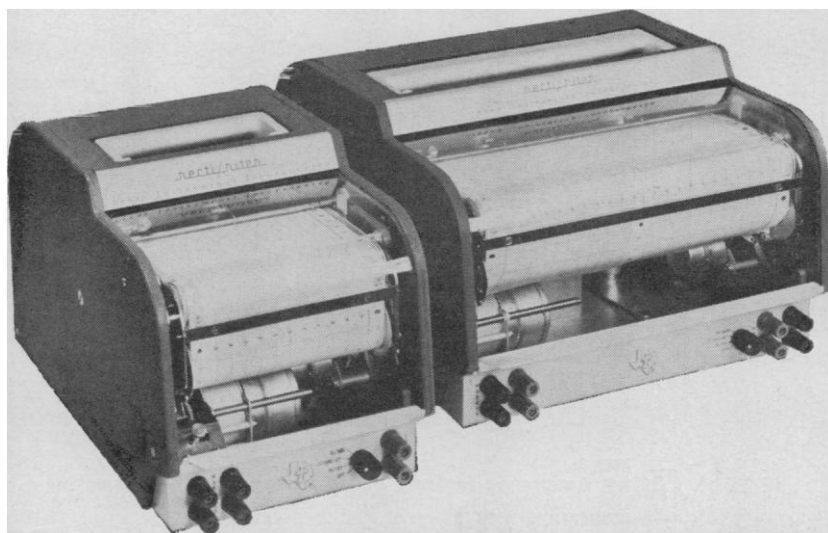
working areas are selected a simple filter instrument probably can be more sensitive if similar conditions are preserved.

The fluorescence of several homologous series of derivatives of indoles, sulfonamides, pyridoxines, and related compounds was analyzed over a range of hydrogen ion concentration by Williams (St. Mary's Hospital Medical School, London). He showed how electronegativity or positivity of substituent groups at various positions on the aromatic ring can be used to predict whether a new member of the series will be fluorescent or not. James (St. Mary's) described several methods for measuring plasma and urinary steroids in man which provide the basis for clinical research, diagnostic tests, and control of therapy. The methods for plasma generally involved simple extraction and assay on a filter fluorometer, but urinary tests had to be run on a spectrophotofluorometer to obtain the necessary specificity.

Corticosteroid and estrogen methods are applicable to normal levels in plasma and urine by nature of the sub-microgram sensitivity of the fluorescence assay. Separation on paper or columns is necessary to identify specific estrogens. It was pointed out that there is a method for converting non-fluorescent androgens to fluorescent estradiol by the use of a placental enzyme to introduce the hydroxy and aromatize the "A" ring. The resultant product in ethanol and concentrated sulfuric acid is fluorescent enough to measure 0.1 microgram per 100 milliliters.

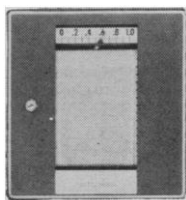
With simple solvent extraction and measurement in ethanol- H_2SO_4 , cortisol output in urine can be measured when Dexamethasone, a synthetic nonfluorescent steroid, is given to suppress cortisol secretion. Tests of pituitary response, adrenal response, and hepatic function in clearing the plasma were described, and their potentialities in diagnosis and therapy were pointed out.

Several methods for enzyme assay based on the release of a fluorescent product from a suitable synthetic substrate were reviewed by Roth (Hôpital Cantonal, Geneva). The method for determining trypsin with an arginine β -naphthylamide substrate was described as a clinical procedure applicable to the assay of trypsin in pancreatic juices. Another procedure also releasing the highly fluorescent β -naphthylamine utilizes leucine naphthylamide for plasma leucine amines and may



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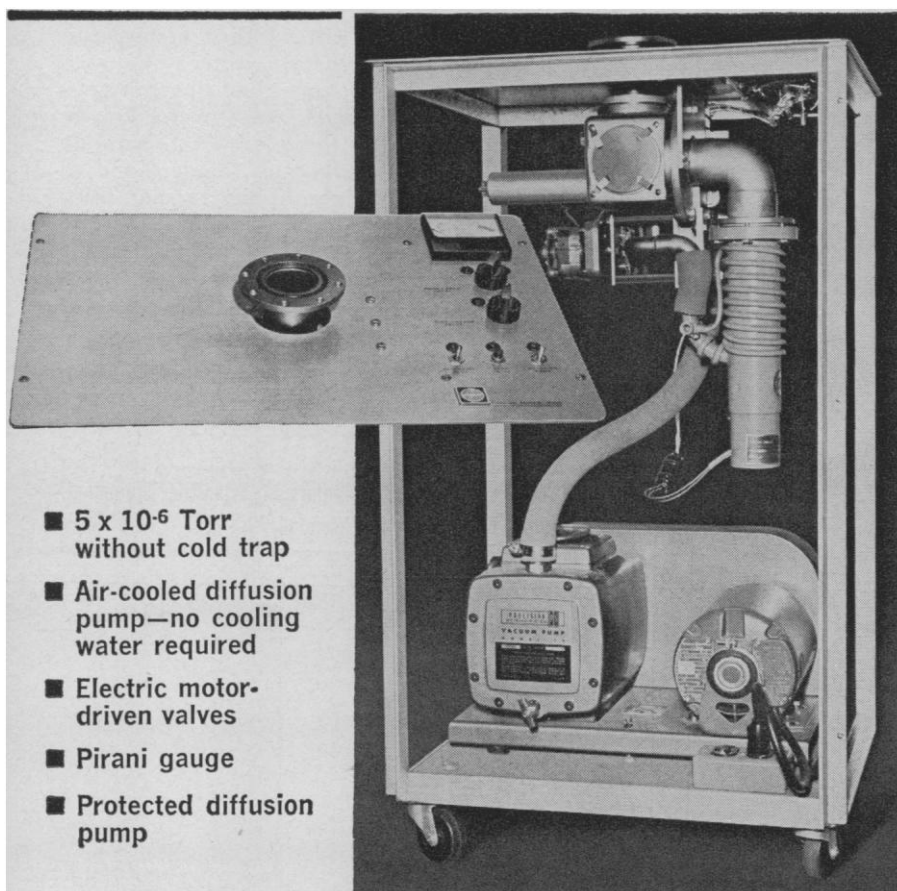
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be of value in the diagnosis of liver or pancreatic disease. Other synthetic substrate systems with fluorescent products and a method of coupling a dye to the product for histochemical localization of enzymes were reviewed.

Falck (University of Lund, Sweden) showed some of his remarkable fluorescence photomicrographs of the monoamines in adrenergic tissue. With this method it is possible to produce clear pictures of the amines stored in the adrenergic nerve endings. When the tissue is prepared by freeze-drying and then is exposed to dry formaldehyde gas, a highly fluorescent product is formed with green fluorescence from noradrenaline and dopamine and yellow-green fluorescence from serotonin. Reserpine depletion experiments and assay by other techniques have confirmed the specificity and resolution of the method. In ganglia, the terminations of adrenergic fibers on cell bodies showed up as sharply defined regions surrounding the relatively clear cell bodies of the neurons.

The value of fluorescence and phosphorescence methods in determining molecular structure was illustrated by Parker (Admiralty Materials Laboratory, Poole, England). He pointed out the possibility of converting fluorescence to phosphorescence and utilizing energy transfer systems for selective quenching of interfering substances. Emphasis was placed on the quenching effect of oxygen and the greater effect for longer duration of the excited state, Schwartz (Hoffman-LaRoche, Basle) reviewed methods involving dehydrogenation of tetrahydroisoquinolines to form fluorescent products by treatment with mercuric acetate-acetic acid reagent for the determination of several important alkaloids in tissues. Van-Duuren (New York University Medical Center) analyzed curves showing how the ratio of dye to nucleic acid can affect the wavelength of the fluorescent peaks of the dye and thus indicate the form of the aggregation of the dye on the nucleic acids. He also showed how fluorescence spectra of polycyclic aromatics could be obtained from material incorporated into potassium bromide pellets. Spectra of charge transfer complexes that may be useful in the study of the carcinogenic activity of these substances were obtained by this method.

The details and practical suggestions for the assay of tissues for catecholamines and related compounds were presented by Magnusson (Göteborg, Swe-

den), and the chemistry of the compounds was discussed by Werdinius (Göteborg). The method requires great care, and various laboratories find different modifications necessary for optimal results. R. L. Smith (St. Mary's) presented the modifications necessary for application to adipose tissue.

In a review of the pharmacology of the catecholamines, Costa (National Institutes of Health) presented a working theory of the interaction of the mediators, monoamine oxidase, the inhibitors, reserpine, and other drugs. The theory provides a good picture of the current state of the art. Spectrofluorometric techniques are used in much of this work.


Bowman concluded the lecture sessions with some discussion of new techniques utilizing low-energy electrons to excite fluorescence and suggested that new advances in fluorescence techniques are just as likely to develop from experimentation as from analysis of the complex theoretical possibilities.

The institute is now examining the possibilities of holding another session within the year for the more than 100 qualified applicants who could not be accommodated in this session.

ROBERT L. BOWMAN
*National Institutes of Health,
Bethesda 14, Maryland*

High Magnetic Fields: Production and Application

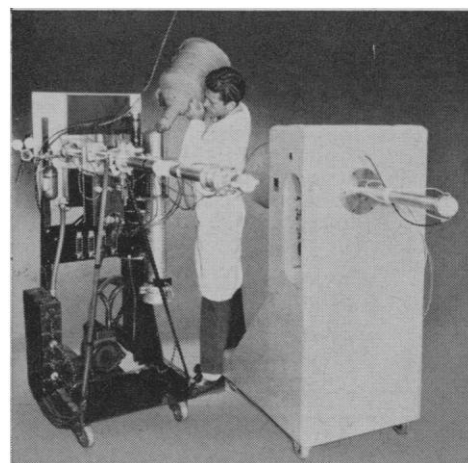
In the evolution of research, cross divisions of science sometimes arise which are based on specialized objectives or experimental techniques. Such research areas cut across the time-honored, historic, or pedagogic divisions of science and are often reabsorbed into these after a more or less brief period of vigorous progress and cross-fertilization. The field of low-temperature physics has enjoyed such a transient existence during the past decade or two, and high magnetic-field research, a closely related area, appears to be following. The environment of low temperature and high magnetic field actually have a great deal in common. Both are of value largely because they are conducive to minimum entropy, and each environment is helpful, if not necessary, in generating the other. The community of high magnetic-field research had its formal birth at the first international conference, held at Mas-



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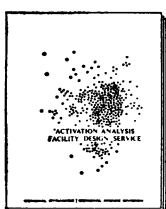
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