in phenol/water, showed fluorescent areas comparable to those obtained with partial hydrolyzates of ACTH protein (*3*), possessing adrenocorticotropic activity.

Intact ACTH protein at a level of 2 mg to 4 mg was then run on paper in phenol/water, as a control, and several spots giving a strong ninhydrin color, together with two distinct fluorescent areas, were observed (Fig. 2). Both fluorescent areas, when eluted, showed adrenocorticotropic activity, while the rest of the paper showed minimal amounts. The fluorescent area of R_f value 0.95 was cut out from the paper and its nitrogen content determined. It was found to contain about 15% to 20% of the nitrogen that was originally placed on the paper.[1]

Tyslowitz (*6*) has reported that phosphotungstic acid will precipitate the activity found in ultrafiltrates of hog pituitary extracts. We have found that phosphotungstic acid supernatants of ACTH protein showed no activity and no fluorescent areas when run on paper in phenol/water.

Dialysis of ACTH protein was performed in distilled water, and against buffers of pH 3 and pH 9, and in 4M urea solution. Twenty mg of the protein was dialyzed in Visking sausage casings in the cold (5° C), for three days against 10 volumes of bathing fluid. The dialyzates in all such experiments showed marked activity. When distilled water was the bathing fluid, for example, a minimum of from 7% to 16% of the original activity could be demonstrated to have passed through the membrane. In such experiments 15% to 20% of the original nitrogen is dialyzable.

We believe that in all the experiments we have performed and reported here the conditions were not such as to lead to the hydrolysis of peptidic linkages. Whether an ACT polypeptide exists in the free state in both the pituitary gland and in sheep ACTH protein hormone, perhaps being adsorbed on the major protein constituent of the hormone, whether there exist both a free peptide and a protein, both of which possess adrenocorticotropic activity, or whether some linkages other than peptidic allow association-dissociation of an active polypeptide and the protein, is not known at the present time. However, if such association-dissociation does exist, it is difficult to understand how single electrophoretic peaks could be obtained over the range of pH from 2.2 to 7.0 (*2*), when it has been shown here that dialyses at pH 3, and against distilled water, along with the 4M urea dialysis, all led to activity in the dialyzate. On the other hand, the inadequacies of electrophoretic analyses of proteins containing small amounts of polypeptide material must be borne in mind. These problems are being investigated at the present time.

Similar considerations to these have been applied to the controversy concerning the protein nature of the posterior pituitary hormones (*1*).

[1] Further experiments performed on additional ACTH protein preparations since this paper was first submitted for publication have shown that not all such preparations give a pattern similar to that shown in Fig. 2. In such cases the TCA supernatant does not show increased activity per unit nitrogen.

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Use of a Wire Recorder for Recording Geiger-Müller Pulses[1]

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In certain medical research studies involving the use of radioisotopes as tracers, it is necessary to perform the studies at two or more locations simultaneously. To have on hand the necessary counting and recording equipment would be both expensive and difficult, particularly since special recording equipment is needed to get the desired result from these studies. The simplest and most inexpensive method of solving this problem has been through the use of the commercial wire recorder. To use such an instrument it is necessary only to provide a power supply for the Geiger-Müller tube, the output of which can be fed into the low gain input of any commercially available wire recorder (e.g., the Webster Model 78).

After the recording is made it is played back in the conventional manner through a pulse limiter and the usual Geiger-Müller counter amplifier, pulse-shaping circuit, and scaler. The end result is exactly like that obtained by using the Geiger-Müller counter directly. The result can easily be checked and adjusted by the use of an oscilloscope which shows the pulse height and width.

The wire recorder affords many other advantages. The wire can be replayed if a check of the result is desired. The same wire can also be used to record voice information pertinent to the experiment in progress. This makes it possible for only one operator to perform the experiment and record the data. At a later time, one person can easily remove all the information on the recorded wire under more favorable conditions than might exist at the time of the original experiment.

With the proper auxiliary equipment it is also possible to record simultaneously on the same wire the counts from two separate Geiger-Müller tubes. This has been tried several times with very good results. A timed switching circuit is employed which switches the output of one of the Geiger-Müller counters on and off at regular timed intervals of 30 sec–1 min. The end result is a record on the wire of the counts from first one tube and then both tubes together. Over the period of time needed for the experi-

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ment this will average out so that the result from each tube can be found by simple subtraction.

This system has the advantage of simplifying the operation as compared with use of the conventional counting systems. In addition, only one expensive counter amplifier, scaler, and chart recorder is needed to carry on work at several locations simultaneously. In the studies performed in our laboratories, it has been necessary to obtain a record of the counting rate for each minute throughout a 15–20-min period (*1, 2*). This requires a rather elaborate computer and chart recorder. A considerable financial saving and an increase in operating efficiency have been effected by the use of several wire recorders and only the one counter amplifier, computer, and chart recorder.

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Ultraviolet Microscopy and Microspectroscopy of Resting and Dividing Cells: Studies with a Reflecting Microscope[1]

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Recent advances in microscopy, including the development of achromatic and apochromatic reflecting optics (*7, 9, 21, 22*), make it possible to study many fundamental biological problems in a manner not heretofore possible (*2, 3*). In this investigation are described the methods and results of a study of living cells with a reflecting ultraviolet microscope. The limits of monochromatic exposure are estimated in terms of the total ultraviolet radiant energy which does not produce abnormal modification of cells in tissue culture. Ultraviolet microspectroscopy of living cells is carried out within those limits, but subject to the consideration that the extinction values may be affected by one or more of the following factors: (1) the absolute amount of absorbing materials (*12*); (2) the volume in which absorbing materials are distributed (*30*); (3) the loss of light by dispersion or refraction (*12, 30*); (4) the spatial orientation of absorbing materials (*13, 17*); (5) photochemical reactions which may accompany the absorption of light quanta (*25*) during image formation; and (6) chemical changes associated with the oxidation or reduction of absorbing materials themselves (*16*).

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[2] Fellow in Cancer Research of the American Cancer Society recommended by the Committee on Growth of the National Research Council.

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METHODS

Optical System. The optical system of the reflecting microscope[4] consists of an objective and a condenser, which are two identical lenses of a reflecting-refracting type (*21*). The numerical aperture is 0.72; the focal length is 2.8 mm; and the magnification is 53×. The objective is used alone or with a 4× amplifying-type eyepiece.

Light Source. The ultraviolet light source is a mercury arc (Type A-H4) with the outer bulb removed. In the illuminating system[5] the central image of the arc is focused and projected by aluminized mirrors so that the beam uniformly illuminates just the full aperture of the condenser. When both condenser and objective are in focus, the full aperture of the objective is filled with light in accordance with Köhler illumination.

Wavelength. The selection of wavelength is made by a set of monochromatizing transmission filters for the ultraviolet region (*1, 4, 23*). The principal pairs of mercury lines isolated by three filter combinations and observed with a quartz spectrograph are those at 253.7 and 265.2 mμ, at 275.3 and 280.4 mμ, and at 312.6 and 334.1 mμ.

Photographic Method. The ultraviolet images are recorded by the photographic method, which is the most satisfactory method of ultraviolet image conversion available at present (*24*). According to Caspersson (*13*), only the photographic method is useful for the measurement of the absorption spectra of very small (less than 1 μ) and irregular bodies, although its accuracy, of the order of 5%–10%, is inferior to that of photoelectric methods.

The emulsions which have been selected to compromise the mutually opposing requirements of high sensitivity and high resolution in the ultraviolet region are the Kodak 103-O UV (spectroscopic plate) and the Kodak 1372 (35-mm film). The relative speed of Kodak 103-O UV at 260 mμ is about four times that of Kodak 1372. The resolution of Kodak 103-O UV, on the other hand, is about one-third that of Kodak 1372.

Processing of the negatives is carried out under controlled conditions of temperature, time, and technique which give maximum contrast and reproducible gamma.

Microdensitometry. Microdensitometry of the processed negatives is carried out with a photoelectric microphotometer that has a mechanically positioned film and plate carrier, interchangeable illuminating apertures, and a device for viewing and centering an area for measurement. The radius of the illuminating aperture is chosen so that it will be less than or equal to one-third of the radius of the image area (*12*).

[4] Designed by Mr. David Grey of the Polaroid Corporation and constructed by the Bausch and Lomb Optical Company under the supervision of Mr. L. V. Foster (*18*).

[5] Designed by Mr. David Grey and constructed by the Research Department of the Polaroid Corporation.