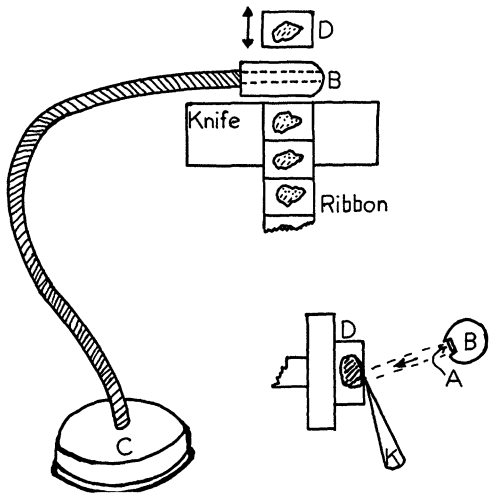

FIG. 1.

time the polonium is plated on the surface. The equipment may be obtained from Mr. Jenkins.

Since alpha rays alone are formed there is no radiation hazard involved from the radiation. Because grease or dirt left on the emitting surface from the fingers will decrease the radiation produced, the polonium strip is protected from contact by being supported within the recess of the head. Should the surface be touched accidentally fingers may become contaminated and should be washed thoroughly with soap and water before eating or smoking, as polonium taken internally is poisonous.

Preparation of Nonprotein Fractions Possessing Adrenocorticotropic Activity from Sheep ACTH Protein

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The possibility that nonprotein material possessing adrenocorticotropic activity may be associated with ACTH protein has been investigated. The stability of the protein hormone to boiling, even in 0.1M HCl, its resistance to destruction by strong solutions of NH_4OH, and the retention of activity even after acid and peptic digestion (*2*), all suggests that the isolated ACTH is indeed an exceptional protein.

Trichloroacetic acid (TCA) precipitation and dialysis have been employed as means of obtaining nonprotein material from an ACTH protein, as prepared by C. H. Li (*2*). All fractions were assayed by the ascorbic acid depletion test of Sayers *et al.* (*4*).

In the usual TCA experiment 20 mg of the ACTH protein was dissolved in 2 ml of water, and 2 ml of ice-cold 10% TCA was added. The solution was stirred and then centrifuged. The supernatant was decanted and the precipitate was suspended in 2 ml of water; 2 ml of ice-cold 10% TCA was again added. The stirred solution was centrifuged, and the supernatant was added to the previous supernatant. The combined supernatants were considered as the TCA supernatant fraction. The precipitate was resuspended and reprecipitated eight to ten additional times, the supernatants being discarded. The final precipitate was washed three times with 4 ml of diethyl ether, and then dissolved in 2 ml of pH 7.5 phosphate buffer. The TCA supernatant fraction was extracted ten times with 4 ml of diethyl ether. The resulting solutions of both the precipitate and supernatant fractions were appropriately diluted, and aliquots were taken for ascorbic acid assay, nitrogen determinations, assay by the repair test of Simpson *et al.* (*5*), and paper chromatography.

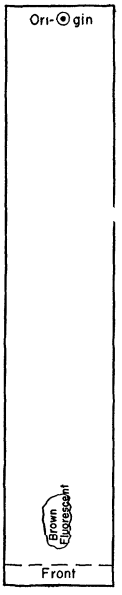

FIG. 1 (left). Ascending chromatogram of a 5% TCA supernatant of sheep ACTH protein run in phenol/water, and developed with a 0.2% solution of ninhydrin in ethanol.

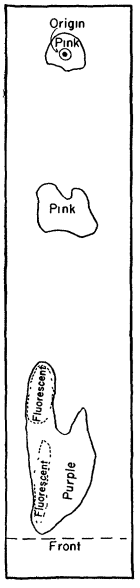

FIG. 2 (right). Ascending chromatogram of 4 mg of sheep ACTH protein run in phenol/water, and developed with a 0.2% solution of ninhydrin in ethanol.

It was found that both the supernatant and the precipitate possessed activity by either assay procedure. By the ascorbic acid depletion method quantitative values were obtained which, when compared to the nitrogen content of each phase, demonstrated that the precipitate contained a smaller amount of activity per unit of nitrogen than the original protein. The supernatant, however, showed a markedly increased amount of activity per unit of nitrogen. In various experiments, utilizing different batches of sheep ACTH protein, this has ranged from six to over ten times that of the original protein. In the various TCA supernatants, from 25% to over 40% of the original activity could be recovered, whereas only 4% of the nitrogen was present in the supernatant fractions. Paper chromatograms of both the TCA precipitate and supernatant fractions (Fig. 1), when run

[1] The authors wish to acknowledge the advice of Dr. John H. Northrop, who was kind enough to read and criticize this paper.

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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