to unactivated ZrP_2O_7 samples.[1] The polonium was (a) added in form of a solution (HCl) and (b) precipitated on ferric hydroxide [$Fe(OH)_3$].

In case (a) the exact quantity of polonium was determined by spinning a nickel foil in an aliquot part of the polonium solution used for the experiment and by measuring this foil by photoelectric or ionization methods. The solution was thoroughly mixed with the zirconium sample and dried at only slightly elevated temperatures until the original weight was reached. Because of the high specific activity of polonium, no correction factor with respect to density changes have to be made. Two series of measurements gave the average value of $\mu d = 2.20 \times 10^{-3}$.

In case (b) a nickel foil with a well-known amount of polonium was dissolved and the polonium precipitated on $Fe(OH)_3$. By this procedure about 5 per cent of the original polonium quantity is lost, and this factor, as well as the change in density by adding approximately 10 mg. of $Fe(OH)_3$ to 1 gram of sample material, was taken into consideration. Six series of measurements gave the average value of $\mu d = 2 \cdot 10^{-3}$; this value is smaller than that in (a) and 11 per cent smaller than in case 1. In three series out of these six we measured only the quantity of added $Fe(OH)_3$ before the precipitation with polonium and did not observe the weight after having dried the sample. It might be that, because the drying was not complete, the value of density appears too small. On the other hand, the values of series 1 may be too high, as the samples were very dilute with respect to protactinium, so that the active material is covered with an absorption layer.

The average value of the three sets of measurements, $\mu d = 2.22 \cdot 10^{-3}$, was accepted and introduced in Equation 3, which now allows us simply to calculate the content of protactinium precipitated on ZrP_2O_7, if the sample is measured in alpha-saturated layers.

For routine measurements, the necessary calculations can be still further simplified by comparing the ionization above the protactinium samples with that appearing over the uranium standard. If the ionization effect is the same in both cases, assuming equal surfaces of the preparation, we find that the content of protactinium in the zirconium sample is 1.3×10^{-5} gram/gram of sample. Therefore, the protactinium content in grams per gram of sample can be expressed by

$$Pa = \frac{J_{ZrP_2O_7+Pa}}{J_{U_3O_8}} \cdot 1.3 \cdot 10^{-5} \text{ gram.}$$

The above considerations, of course, are valid only if the radioactive element is always separated by the same chemical processes, a common occurrence in routine work of this nature.

The use of polonium for the determination of the factor μd in the case of protactinium samples is self-indicating because of the nearly equal range of the alpha particles emitted by these radioactive elements. However, the same procedure can be recommended in the case of other alpha emitters of appreciable half-life, e.g. plutonium and other transuranic elements, since the addition of polonium, due to its high specific activity, does not alter the absorption in the sample.

Reference

1. Evans, R. D. *Phys. Rev.*, 1934, 45, 29.

[1] We are grateful to W. R. Horn and M. Pavey, of International Rare Metals Refinery, Inc., for the preparation of all of these samples.

A Simple Quantitative Method for Intravenous Injection of Small Volumes of Fluid

Henry L. Barnett and Francis X. Fellers[1]

New York Hospital and *Cornell University Medical College*
Department of Pediatrics

The intravenous injection of small, accurately measured quantities of solutions is frequently required in physiological investigations. In most of the reported studies the technic has consisted of using a syringe calibrated to contain the solution and final rinsing of the syringe with blood several times to insure complete delivery (*1*). In the course of a study of the distribution of radioactive and nonradioactive substances injected intravenously into small infants, a simple technic was adopted which has several advantages.

The arrangement of the apparatus, which consists of two ordinary glass syringes with Luer locks and a metal three-way stopcock,[2] is shown in Fig. 1. One or more solutions are delivered from sterile volumetric pipettes of desired capacity into the upright syringe barrel, A, with the stopcock handle, B, in position 1. After the vein is entered, the handle is turned to position 2, and the plunger of the horizontal syringe, C, is withdrawn, bringing the solution into the barrel. Returning the handle to position 1 and advancing the plunger into the barrel delivers the solution through the needle into the vein. The upright barrel can then be filled with saline to the height of the original fluid level and washed through the apparatus as many times as desired by repeating the above maneuvers.

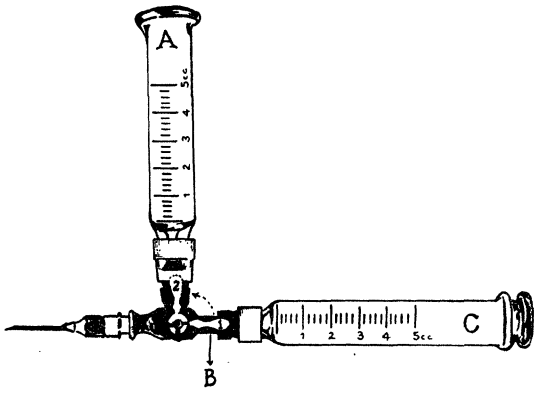

Fig. 1

One-ml. aliquots of four different solutions (thiocyanate, creatinine, mannitol, and sodium-*p*-aminohippurate[3]) were pipetted into the upright barrel of the assembly and then delivered through the apparatus into 2,000-ml. flasks. With two 4-ml. rinsings of the assembly into the flasks, concentrations of the four substances were found to be identical with those in flasks into which similar aliquots were delivered

[1] With the technical assistance of Dorothy Weber.

[2] This is the type commonly used for intravenous injections in pediatrics and is available from Becton-Dickinson & Company, Rutherford, New Jersey.

[3] The mannitol and sodium-p-aminohippurate were kindly supplied to us by W. P. Boger, of Sharp & Dohme, Inc., Philadelphia.

directly from the pipettes. Quantitative recovery was confirmed by finding no residual substances in additional rinsings of the assembly.

The difficulty of intravenous injections in small infants is not increased by using the arrangement of the syringes described. The technic has the following advantages:

(1) The volume or volumes of solution to be injected can be measured more accurately from volumetric pipettes than from calibrated syringes, and the former are more readily available.

(2) Several solutions can be measured quantitatively and given in a single intravenous injection.

(3) Rinsing is done with saline rather than aspirated blood. The small amount of additional fluid required to rinse the assembly will not, in most instances, introduce a significant error in the determination of fluid "spaces."

The last two items are particularly applicable to studies of infants or small animals in which multiple injections and rinsing with blood are difficult.

References

1. KALTREIDER, N. L., MENEELY, G. R., ALLEN, J. R , and BALE, W. F. *J. exp. Med.*, 1941, **74**, 569–590; PACE, N., KLINE, L., SCHACHMAN, H. K., and HARFENIST, M. *J. biol. Chem.*, 1947, **168**, 459-469.

A Crystalline Pituitary Protein With High Growth Activity[1]

JACOB B. FISHMAN, ALFRED E. WILHELMI, and JANE A. RUSSELL

Department of Physiological Chemistry, Yale University

During current work on the fractionation with alcohol of calcium hydroxide extracts of fresh beef anterior pituitary glands we have obtained crystalline fractions with very high growth hormone activity. The procedure is briefly as follows: Dissected anterior lobes are frozen in solid carbon dioxide and ground to a fine powder. After the carbon dioxide has evaporated, the gland powder is suspended in calcium hydroxide solution, pH 11.5, and stirred vigorously overnight. This and all subsequent operations are carried out in a cold room at 0–5° C. The pH of the extract is adjusted to 8.7 by bubbling in carbon dioxide gas, and after the mixture has stood again overnight it is centrifuged and the residue is discarded. To the supernatant solution, vigorously stirred, 1:1 alcohol-water is slowly added. Successive additions yield 5 fractions: A, at pH 8.6 and 13 per cent alcohol; B, pH 8.6, 19 per cent; C, pH 6.8, 23 per cent; D, pH 4.6, 23 per cent; and E, pH 4.6, 40 per cent. The fractions are separated by centrifugation, resuspended in water, and lyophilized. To prepare crystalline material, fraction A or B is dissolved in calcium hydroxide, pH 11.5, to make a 0.5 per cent solution, the pH is adjusted to 8.6 with carbon dioxide, the solution is centrifuged, and to the supernatant solution, vigorously stirred, enough 1:1 alcohol is very slowly added to make an alcohol concentration of 7 per cent. The crystals, which impart a beautiful silken sheen to the solutions from which they appear, seem on microscopic examination to be very thin, rectangular plates, many of them broken because of the shearing stresses of the vigorous stirring which is necessary during the addition of the alcohol. They are centrifuged off, suspended in water, and lyophilized. In the first experiment, starting with 304 grams of fresh glands, the yield of crystalline material from a portion of fraction B was 200 mg. In the second experiment, in which 350 grams of fresh glands were used, the yields were 74 mg. from fraction B and 950 mg. from fraction A.

The three crystalline fractions so far obtained have been assayed by the 10-day growth test on hypophysectomized rats. Their activities were compared with that of a purified growth hormone preparation made by us according to the method of Li, Evans, and Simpson (*1*). The results are summarized in Table 1.

TABLE 1

Fraction	No. of rats	Dose/day		Mean weights of rats (grams)		
		(mg.)	(mg. N)	Initial	Final	Change
30A	3	0.020	0.0032	194	212	+18
49A	3	0.100	0.0147	92	117	+25
	3	0.010	0.0015	95	108	+13
50A	3	0.100	0.0142	91	112	+21
	3	0.010	0.0014	87	98	+11
Li	2	0.099	0.0131	95	120	+25
	3	0.010	0.0013	91	108	+17

Autopsies carried out on the test animals showed that the weights of the thyroids, adrenals, testes, seminal vesicles, prostate, and liver were not different from the weights of these organs in uninjected controls. Histological studies on the thyroids are not yet complete. In the tests with fractions 49A, 50A, and the Li preparation the widths of the tibial epiphyseal cartilages were also measured. These were in μ: for 7 controls, 125; for 49A, 50A, and the Li preparation, at the 0.1-mg. dose level, 320, 309, and 379, respectively; and at the 0.01-mg. dose level, 291, 270, and 278, respectively. These results provide an additional measure of the activity of the crystalline preparations.

The three crystalline fractions have been examined electrophoretically in phosphate buffer (ionic strength, 0.2) at pH 8.0. Two components were noted in each instance. Additional electrophoretic studies on recrystallized material are in progress, experiments are under way to determine whether the two components can be separated, and further work is being done to determine the maximum yield of active material obtainable by the new method.

The results so far indicate that crystalline preparations comparing favorably in growth activity with the purified hormone can be prepared in excellent yields by a relatively simple alcohol fractionation of an alkaline extract of anterior pituitary glands. Although not all of the fractions obtained have been studied thoroughly, it is hoped that the procedure may lead to the isolation in pure form of other active principles of the anterior pituitary gland.

Reference

1. LI, C. H., EVANS, H. M., and SIMPSON, M. E. *J. biol. Chem.*, 1945, **159**, 353.

[1] The work reported in this paper is being done under a grant from the American Cancer Society on the recommendation of the Committee on Growth, National Research Council.