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.