Technical methods

Control material for oxalo-acetic transaminase determination

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The estimation of serum transaminase activity is routinely performed in many laboratories, and, although serum of high activity has been recommended as control material, we have found it of limited value owing to instability for any period longer than a few weeks and because of vagaries of supply. We felt, therefore, that a more stable material was required to control these determinations over longer periods. With this end in view we prepared extracts of cardiac muscle according to the method of Cammarata and Cohen (1951) as far as precipitate WFT-11, omitting the final stages of purification, and studied its activity under varied conditions of storage.

The final yield amounted to approximately 1.0 g. of precipitate from 1,000 g. of wet heart muscle. The precipitate was prepared for storage in two forms. (1) Ampoules were made containing 28 mg. of freeze-dried material each, which was reconstituted with 20 ml. distilled water (1 in 714 wt./vol.) giving a solution with a transaminase activity of 60 units/ml. (2) Aqueous solutions with various convenient levels of enzyme activity were made from the precipitate and these were stored as below.

STORAGE OF EXTRACT

The following conditions of storage were studied:—

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1 Container, polystyrene or glass (Table I A); 2, concentration of extract, high or low (Table I B); 3, temperature, frozen ($-20^{\circ}\text{C}.$), cooled ($+4^{\circ}\text{C}.$), and room (Table I C); 4, freeze-dried and stored at room temperature and reconstituted with water before use (Table II).

TABLE II

RESULTS FOR FREEZE-DRIED EXTRACT

Weeks	Activity as Percentage of Original
0	100
4	94
16	100
20	100
26	94
36	102

Using Sigma prepared reagents and subsequently the method of Reitman and Frankel (1957), the oxalo-acetic transaminase activity was determined at frequent intervals on the material extracted. A selection of the relevant results is shown in the accompanying tables. All results are expressed as a percentage of the original activity.

DISCUSSION

Cammarata and Cohen observed deterioration of activity over a six-week period with their final lyophilized enzyme, but using the less purified preparation we obtained a stable product for at least nine months when it was stored in the lyophilized state at room temperature.

Extract of cardiac muscle was found to be suitable, for approximately six months, as control material for oxalo-acetic transaminase determinations when stored in polystyrene containers at high concentrations at -20°C

TABLE I

RESULTS AFTER STORAGE

Weeks	Activity as Percentage of Original						
	A Both 4,000 units/ml. at $-20^{\circ}\text{C}.$		B Both in Polystyrene at $-20^{\circ}\text{C}.$		C All 8,000 units/ml. Polystyrene		
	Polystyrene	Glass	8,000 units/ml.	40 units/ml.	$-20^{\circ}\text{C}.$	$+4^{\circ}\text{C}.$	Room Temperature
0	100	100	100	100	100	100	100
1	96	96	94	100	94	94	77
2	98	94	94	72	94	94	66
4	100	86	102	47	102	100	49
16	96	70	94	—	94	100	—
26	90	64	102	—	102	70	—
36	78	60	77	—	77	—	—

Freeze-dried extract stored at room temperature is suitable for at least nine months.

We should like to thank Dr. P. J. Sewell for his help with the freeze-drying of the extract.

REFERENCES

- Cammarata, P. S., and Cohen, P. P. (1951). *J. biol. Chem.*, **193**, 53.
Reitman, S., and Frankel, S. (1957). *Amer. J. clin. Path.*, **28**, 56.

The extraction of Evans blue from plasma

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It is possible to extract Evans blue quantitatively from plasma by the following procedure. Unfortunately, haem pigments and the yellow pigments of plasma are also extracted and make a substantial contribution to the absorption at the wavelength of the Evans blue absorption band but this can be corrected by a blank determination.

Evans blue forms a complex with benzalkonium chloride which can be extracted from the aqueous phase with *n*-butanol. Proteins are precipitated and after centrifugation form a tough layer at the interface. Since water and *n*-butanol are mutually soluble, the benzalkonium reagent is saturated with butanol, and kept at + 4°C., thus ensuring that even at the end of the extraction process the temperature is slightly below ambient. If during centrifugation the temperature is allowed to rise above ambient on cooling the extract separates out into two phases and becomes turbid.

REAGENTS

Fifty per cent benzalkonium chloride U.S.P. (Marinol, Bayer Products) is diluted 1 in 8 with distilled water; a drop of concentrated hydrochloric acid is added to each 100 ml., and enough *n*-butanol to saturate it at + 4°C., approximately 8 ml. per 100 ml. This reagent and *n*-butanol (A.R.) are kept in a refrigerator at 4°C.

PROCEDURE

This procedure is designed for the Unicam SP 350 or Coleman junior absorptiometer or other instrument requiring 2.5 to 2.8 ml. samples.

Place 5 ml. benzalkonium reagent in a centrifuge tube and add 2 ml. dyed plasma and 3 ml. *n*-butanol. Cover with Parafilm, shake vigorously, and centrifuge at 3,000 r.p.m. for four to five minutes. Transfer the clear supernatant to a clean tube or directly to the cuvette, and read against a *n*-butanol blank at 605 m μ .

BLOOD VOLUME DETERMINATIONS

It is essential to prepare a blank from a sample collected before the dye is injected. It is not possible to correct by using measurements at 405 m μ , or to remove the interfering pigments.

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