and spectrophotometric methods[2,3]. The major components were pelargonidin 3 : 5-diglucosides acylated with caffeic or *p*-coumaric acid. A minor component was cyanidin 3 : 5-diglucoside acylated with caffeic acid. The concentrations of the anthocyanins in these tissues were determined spectrophotometrically in methanol containing 1 per cent hydrochloric acid, and the results (Table 1) were expressed as mg of pelargonidin 3 : 5-diglucoside using a molecular extinction coefficient of 15,300 at 508 mμ.

Table 1. Effect of foliar applications* of 2,2-dichloropropionic acid ('Dalapon'), trichloroacetic acid (TCA), and 2,3-dichloroisobutyric acid (DCIB) on the concentration of anthocyanin in flowers of *Salvia splendens* cultivar America

Acid equivalent of herbicide (p.p.m.)	mg Pelargonidin 3 : 5-diglucoside/g dry weight 'Dalapon'	TCA	DCIB
0	92	89	91
900	24	73	43
3,000	10	38	24
9,000	+	24	10

* Applied at the equivalent volume of 40 gallons per acre.
+, Phytotoxic

Foliar applications of aqueous sprays of 'Dalapon', DCIB, and TCA were all effective in reducing synthesis of anthocyanins. The reduction in the anthocyanins was directly proportional to the concentration of the herbicides applied to the foliage and flower colour was changed from the original deep reddish orange to almost white. The highest concentration (9,000 p.p.m.) of 'Dalapon' was phytotoxic. The growing point was killed and severe foliar damage was evident. Malformed flower buds were present on plants treated with the highest concentration (9,000 p.p.m.) of DCIB or TCA. The subtending floral bracts became desiccated and florets failed to expand. Lateral flower buds that developed after treatment also showed a reduction in anthocyanin synthesis but not as pronounced as the treated terminal flower buds. Anthocyanins in flowers from treated and untreated plants were identical and, therefore, difference in colour was due to quantitative, not qualitative, changes. Antagonism of the herbicides by foliar applications of calcium pantothenate[4] was not confirmed. The colour of the flowers on plants grown from seed collected from the treated plants was normal and showed no carry-over effect.

We conclude, therefore, the 'Dalapon', DCIB, and TCA interfere with anthocyanin synthesis and that these herbicides may offer a valuable tool for investigations dealing with the biosynthesis of these pigments.

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Isolation and Characterization of Spleen Acid Deoxyribonuclease

In recent years several methods have been described for the partial purification of spleen acid deoxyribonuclease (DNase)[1–4] (see also ref. 5 for a critical review). The isolation of the enzyme in a pure form was considered to be of interest, particularly in view of the results obtained in an examination of the kinetics of the enzymatic degradation of deoxyribonucleic acid[6]. The preparation procedure as well as a preliminary physical and chemical characterization of the enzyme are reported in the present communication.

The preparation of the enzyme was carried out at 4° C. using hog spleen as the starting material. In a first stage a crude enzymatic preparation was obtained essentially according to a procedure[7] involving the following steps: (1) homogenization and extraction of spleen tissue with 0·15 M sodium chloride–0·02 M calcium chloride; (2) precipitation of the clarified extract with ammonium sulphate; the precipitate collected between 34 and 100 per cent saturation was redissolved in distilled water; (3) acidification to *p*H 2·5, to precipitate hæmoglobin, and centrifugation; (4) precipitation of the supernatant from the preceding step with ammonium sulphate; the fraction obtained between 40 and 80 per cent saturation was dissolved in distilled water, clarified by centrifugation, freeze-dried, and stored at −30° C. The crude enzyme was obtained in a yield of about 1 g/kg tissue; it contained about 55 per cent of the acid DNase activity present in the clarified homogenate and its specific activity was 3·0. Both neutral and acid ribonuclease (RNase) activities[8] were present, the ratios DNase/RNase being 3·6 and 1·8, respectively. In all cases activities were measured by determining the optical density at 260 mμ of acid-soluble nucleotides[9] released by enzymatic action under suitable experimental conditions.

The second stage involved the following chromatographic steps: (1) *DEAE-cellulose:* the enzyme was washed out with 0·005 M phosphate buffer *p*H 8·0; the total activity was about 10 per cent higher after chromatography, probably because of the adsorption of an inhibitor on the column; the adsorbed protein, containing most of the RNase activity, could be eluted with 0·15 M acetate buffer, *p*H 5·0; (2) *hydroxyapatite:* elution was carried out with a linear gradient of phosphate buffer *p*H 6·8, of molarity increasing from 0·05 to 0·5; the enzyme was eluted immediately after a bright red fraction, tentatively identified with cytochrome *c*; (3) *hydroxyapatite*: elution was performed as above, the enzyme being removed from the column at about 0·2 M phosphate; (4) '*Amberlite IRC-50*': the enzyme was loaded in 0·1 M phosphate buffer *p*H 6·0; some inactive protein was not retained on the column; the enzyme was eluted with a linear gradient of phosphate buffer *p*H 6·0, 0·1–0·5 M; a subsequent gradient of 0·5 M phosphate buffer *p*H 6·0–8·0 eluted more inactive protein. Rechromatography of the enzyme on 'Amberlite *IRC-50*' gave a symmetrical peak of constant specific activity equal to 210 ± 10 (Fig. 1); no acid or neutral RNase activity was detectable in this product. About 25 per cent of the enzymatic activity present in the crude enzyme was recovered after the final rechromatography.

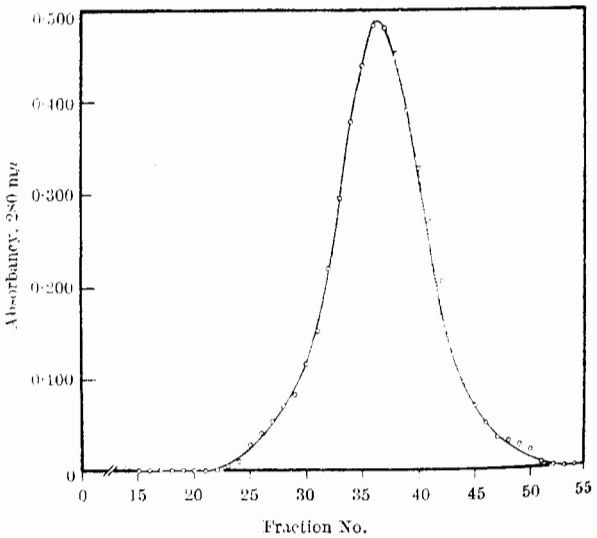

Fig. 1. Rechromatography of hog spleen deoxyribonuclease on 'Amberlite *IRC*-50' (2 cm × 12·5 cm). Elution was carried out at 4° C with a gradient of phosphate buffer *p*H 6·0, 0·1–0·5 M. No optical density was eluted with a subsequent gradient of 0·5 M phosphate buffer from *p*H 6·0 to 8·0. Fractions of 5 ml. were collected

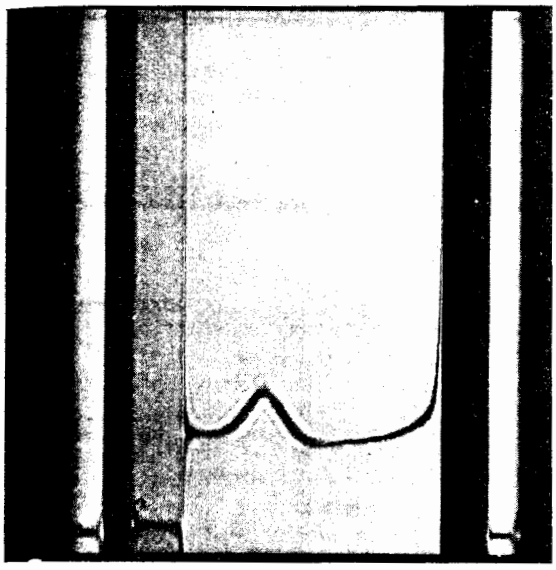

Fig. 2. Sedimentation pattern of hog spleen deoxyribonuclease in 0·15 M acetate *p*H 5·0. Exposure taken after 56 min at 59,780 r.p.m.

An ultracentrifuge analysis showed that the enzyme sedimented as a single boundary in 0·15 M acetate buffer *p*H 5·0 (Fig. 2). No dependence on concentration was observed and the sedimentation coefficient was $S^0_{20,w} = 3{\cdot}4\ S$. The same value was obtained in acetate buffer *p*H 3·0, $\mu 0{\cdot}1$, and phosphate buffer *p*H 7·8, $\mu 0{\cdot}1$. In glycine buffer *p*H 8·9, $\mu 0{\cdot}1$, the sedimentation coefficient was found to be 4·0 *S*, and a small amount of a faster component was present. In glycine buffer *p*H 2·0, $\mu 0{\cdot}1$, the sedimentation analysis indicated extensive aggregation.

Electrophoretic runs were performed on celluloso acetate strips at 6 V/cm. Only a single band was evident at four different *p*H values ranging from 4·6 to 9·2. By extrapolating the mobilities obtained as a function of *p*H to zero mobility, an isoelectric point close to 10·2 could be calculated.

An amino-acid analysis was carried out, using three different times of hydrolysis. Neutral and acidic amino-acids were predominant; the amount of ammonia (as estimated after extrapolation to zero time of hydrolysis) was consistent with most or all of the acidic amino-acids being in the form of the corresponding amides, a fact which would explain the high isoelectric point.

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Changes in Rat Plasma and Serum Calcium during Storage

PREVIOUS to conducting a clinical field experiment it became necessary to determine how components of the (circulating) blood, serum and plasma, would withstand storage and remain suitable for accurate and reproducible calcium analytical data.

Serum and plasma calcium data are somewhat confused as shown by the variety of 'normal values' reported by several authors[1–3] and by the extensive number of 'practical procedures' for the estimation of serum and plasma calcium[4]. In this laboratory many of these calcium micro-procedures have failed in duplicate analyses on the same sample and only one method, that of Munson *et al.*[5], was found to be precise to 0·2 mg per cent calcium. This procedure[5] was used exclusively in all subsequent calcium determinations.

As a rule serum is the fluid generally chosen for determinations of calcium. However, in many instances plasma is equally advocated[2,3,6] and at times, when values for other blood constituents are desired from the same sample (for example, corticosterone-levels), one is restricted to the use of blood plasma.

Mature Sprague–Dawley rats fed a standard laboratory chow and water *ad libitum* were used for this experiment. Whole blood was obtained by cardiac puncture under ether anæsthesia. Heparin sodium was used when plasma was desired. The effect of storage of plasma and of serum at 4° C and −20° C was investigated. All determinations were made by the same technique[5] and the same operator. Each group of samples was divided into four parts for: (*a*) base-line data, based on a fresh specimen of blood, and (*b*) data on specimens stored for 1, 2 and 3 months.

Table 1

	a Plasma (9)* Mean (mg %) *S.E.*	*b* Plasma (9)* Mean (mg %) *S.E.*	*c* Serum (10)* Mean (mg %) *S.E.*	*d* Serum (5)* Mean (mg %) *S.E.*
Fresh sample	9·9 ± 0·06 (Frozen)	10·4 ± 0·16 (4° C)	10·3 ± 0·08 (Frozen)	10·4 ± 0·24 (4° C)
1 month	8·3 ± 0·46	9·5 ± 0·17	10·4 ± 0·09	10·4 ± 0·12
2 months	8·4 ± 0·12	9·7 ± 0·14	10·3 ± 0·08	10·6 ± 0·08
3 months	8·2 ± 0·25	Deterioration of plasma	10·4 ± 0·08	10·5 ± 0·17

* Numbers in parentheses indicate number of individual samples.

The results are presented in Table 1. Analysis of plasma kept in the frozen state or at 4° C demonstrated a significant drop in the calcium-level from the results on the fresh specimen. This did not occur in the serum kept frozen or at 4° C. In order to determine if the low calcium values in the quick-frozen plasma after 3 months could be corrected, 7 additional plasma specimens were obtained, analysed immediately, and after 3 months at −20° C an aliquot of this plasma was ashed and the determination of calcium carried out. The results listed in Table 2 demonstrate that, in the ashed plasma, calcium values were the same as the initial value. As before, an unashed aliquot of the plasma determined after three months showed a decrease in calcium from the initial figure.

Table 2

	Plasma (7)* Mean (mg %) *S.E.*
Fresh sample	9·6 ± 0·16 (Frozen)
Ashed, 3 months	9·6 ± 0·18
Unashed, 3 months	9·1 ± 0·14

* Number of individual samples.

These results indicate that calcium determinations of rat plasma which has been quick-frozen or kept at 4° C for 1 month or longer are not reproducible by the Munson technique[5]. However, by ashing the plasma that had been frozen for 3 months the calcium analysis agreed with the initial determination. However, the serum calcium