2. The determination of tyrosine in gelatins by spectrophotometric and chromatographic methods has been discussed. The former has been considered with special reference to the elimination of interference by certain constituents of gelatins.

3. The tyrosine contents of a number of gelatins have been determined by the three methods. Results are comparable; in addition similar values are obtained from intact and hydrolysed samples of the same gelatins. The gelatins studied contained 0·2–0·8 % of tyrosine.

4. Fractionation of gelatin by alcohol coacervation concentrates impurities into the first and last fractions, which have a considerably enhanced tyrosine content. The intermediate fractions have tyrosine contents that do not exceed that of the original gelatin, no fraction containing less than 0·2 % of tyrosine.

5. The results support the view that a proportion of the tyrosine in gelatins is combined in the protein structure, together with a tyrosine-containing impurity.

The authors wish to thank Dr J. E. Eastoe of the Royal College of Surgeons for making available the original chromatograms of his Moore & Stein analysis, and Dr A. A. Leach of The British Gelatine and Glue Research Association for helpful discussions. This paper is published by permission of the Director and Council of The British Gelatine and Glue Research Association.

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The Form of Vitamin C Released by the Rat Adrenal

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(*Received* 12 *April* 1962)

The adrenal gland of the rat loses part of its very high concentration of vitamin C (ascorbate and dehydroascorbate) under a variety of conditions. ['Dehydroascorbate' is used in this and the next paper as a conventional term for the reversibly oxidized form of vitamin C: it is not meant to imply that 'dehydroascorbic acid' ionizes in water as do ascorbic acid and dioxogulonic acid (cf. Lloyd & Sinclair, 1953).] Slusher & Roberts (1957) reported that the loss of vitamin C by the adrenal gland equals the gain by the adrenal-vein blood, and Salomon (1957, 1958) concluded that ascorbate is not the principal component of this loss and gain. Slusher & Roberts (1957) used the method of Roe & Kuether (1943), which estimates a total of ascorbate, dehydroascorbate and dioxogulonate, and their work therefore did not specify the nature of the material leaving the gland. In the present work parallel estimations have been made of ascorbate [by the method of Mindlin & Butler

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(1938)] and of the total of ascorbate, dehydroascorbate and dioxogulonate [by the method of Roe & Kuether (1943)] in the adrenal gland and in the venous blood from the adrenal. Briggs & Toepel (1958) showed that under some conditions the correlation between the loss by the adrenal gland and the gain by the adrenal-vein blood may be smaller than that reported by Slusher & Roberts (1957). They estimated either ascorbate or the total of ascorbate, dehydroascorbate and dioxogulonate, and their work implies that the loss from the adrenal gland is a loss of ascorbate. The present experiments appear to be the first direct demonstration that ascorbate accounts almost entirely both for the decrease of vitamin C in the rat adrenal and for the increase of vitamin C in the adrenal effluent blood.

EXPERIMENTAL

Materials

Cortisone was supplied as Cortelan, a suspension containing 25 mg. of cortisone acetate/ml., by Glaxo Laboratories Ltd. Corticotrophin was supplied as Acthar by Armour Laboratories, Armour and Co., Chicago, Ill., U.S.A., 25 i.u. being dissolved in 1 ml. of sterile 0·9% NaCl for intravenous injection. Heparin was supplied by Roche Products Ltd. as Liquemin, containing 5000 i.u./ml. Nembutal (pentobarbitone sodium), in a solution containing 60 mg./ml., was supplied by Abbott Laboratories Ltd.

Analytical methods

Ascorbate was estimated by the method of Mindlin & Butler (1938); turbidity was estimated by taking an absorptiometer reading after complete reduction of the dye with solid ascorbic acid. Whole-blood filtrates for ascorbate estimation were prepared by a modification of the method of Deeny, Murdock & Rogan (1942). Coal gas used for displacing oxygen from blood was freed from reducing gas and oxygen by passing it through alkaline pyrogallol and boiled water. Silicone (M.S. Antifoam 'A', Hopkin and Williams Ltd.) was used instead of octanol to prevent frothing.

The ascorbate plus dehydroascorbate plus dioxogulonate was estimated by the method of Roe & Kuether (1943). Charcoal was added before the precipitated protein was removed, and turbidity was estimated by means of readings at 610 mμ. Metaphosphoric acid was used for precipitating the proteins of blood and of adrenal homogenates in the first instance. For estimation by the method of Roe & Kuether (1943) trichloroacetic acid was added to give a final concentration of 4% (w/v).

Animals

Adult male albino Wistar rats weighing 330–430 g. were maintained on pellet diet 41B (cf. Cuthbertson, 1957) and tap water, and kept in groups in cages in one room at about 25°. Before experiments the animals were placed in individual cages for about 18 hr. Slusher & Roberts (1957) obtained similar results with normal and hypophysectomized animals, and normal animals were used in these experiments. It is claimed that the administration of large quantities of corticosteroids inhibits the release of endogenous corticotrophin (cf. Sayers, Redgate & Royce, 1958), and 25 mg. of cortisone acetate was injected intraperitoneally about 4 hr. before the experiment.

Cannulation and collection of blood

In essence, the procedure of Vogt (1948) was followed. Animals were anaesthetized with intraperitoneal Nembutal (the initial dose of 45 mg./kg. was often followed by smaller doses to maintain anaesthesia). Body temperature was maintained with a 200w lamp near the animal. After laparotomy, the left renal vein was cleared of adhering tissues, special care being taken to keep the inferior suprarenal artery and the suprarenal vein untouched. Three loose ligatures were arranged: one was at the junction of the vena cava and the renal vein, avoiding the renal artery, the second was passed close to the kidney round the root of the renal vein and the third lay just central to the second for tying in the cannula. Extra-adrenal tributaries to the left renal vein were ligated. The left carotid artery was also prepared for cannulation. At this time the right adrenal gland was removed and dropped into a weighed tube (1 cm. × 7·5 cm.) containing 2·5 ml. of 0·9% NaCl. This was then placed in solid carbon dioxide.

Heparin (100 i.u./100 g. body wt.) was injected into the right femoral vein. The left kidney was excluded from the circulation by the ligature and the left renal vein cannulated with a piece of polyethylene tubing (about 20 cm. long and 1 mm. external diameter) leading to a collecting tube. The carotid artery was cannulated and blood collected to determine the concentrations in arterial blood of ascorbate and of ascorbate plus dehydroascorbate plus dioxogulonate. The left renal vein was ligated at the junction with the vena cava and 3 i.u. of corticotrophin injected through the right femoral vein. Immediately blood was also allowed to flow from the carotid artery into a collecting tube. In some experiments the flow of arterial blood was so adjusted that nearly equal volumes of arterial and adrenal-vein blood were collected by the end of the experiment; at other times two to four samples of arterial blood were collected over the entire period of the collection of adrenal-vein blood. On the average, a total of 4–5 ml. of blood was finally collected: this is about 1% of the body weight and about 15% of the total blood volume.

The procedure from the beginning of operation up to the start of the collection of venous blood required 12–16 min. Collection of blood was continued for 18–40 min. All collections were made under N_2 into weighed tubes in an ice bath. Haemolysis was minimized by preventing the spread of blood to the sides of the tubes.

After the collection of blood the left adrenal was removed and placed in a tube. The adrenal glands were collected in small glass tubes (of approximately equal and uniform bore, and fitted with a glass piston) and homogenized in 2·5 ml. of 0·9% NaCl. Then 1 ml. portions were put into tubes containing 0·4 ml. of 32% (w/v) metaphosphoric acid, mixed with 2·6 ml. of water and centrifuged after being thoroughly shaken. Ascorbate and ascorbate plus dehydroascorbate plus dioxogulonate were estimated in the supernatant. At the end of some of these experiments, in connexion with another piece of work (Lahiri & Lloyd, 1962), ascorbate was estimated in blood collected from the inferior vena cava at the entrance of the hepatic veins.

RESULTS

The results are given in Table 1 and Fig. 1. When several arterial samples were taken separately during the collection of venous blood, an appropriately weighted mean arterial concentration was calculated.

In mammals the weight of the right adrenal is frequently greater than that of the left, being 10–20% higher in rats (cf. Jones, 1957), but in our animals, in which the right adrenal was removed about ½ hr. before the left, the latter was usually greater in weight [mean difference 2·1 mg. (Table 1)].

The increased ascorbate in the adrenal venous blood could have been due to conversion of dehydroascorbate from the adrenal into ascorbate by erythrocytes in the blood (cf. Lloyd & Parry, 1954). To test this possibility, 5 mg. of dehydroascorbate in 1 ml. of 0·9% sodium chloride was added to 2 ml. of rat blood in two instalments at 10 min. intervals. The whole was incubated at 37° for a total period of 30 min. Ascorbate estimation showed that only 0·03 mg. of the added dehydro-

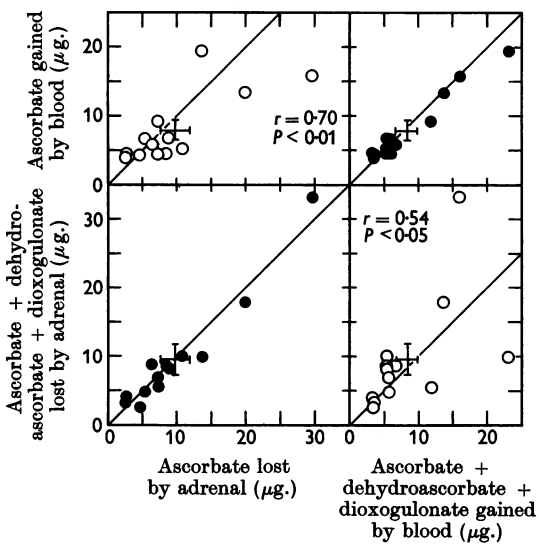

Fig. 1. Nature of the vitamin C leaving the left adrenal of cortisone-treated, anaesthetized, laparotomized, and corticotrophin-treated rats in a condition equivalent to bilateral adrenalectomy. All results are expressed as μg. of ascorbic acid. The scatter diagrams with closed circles (●) show that the material determined by the Roe & Kuether (1943) method lost by the adrenal or gained by the adrenal effluent blood is almost entirely ascorbate. The scatter diagrams with open circles (○) show the correlations between assessed loss by the adrenal and gain by the adrenal blood. Lines of equality are drawn in and means and S.E. values denoted by the crosses.

Table 1. *Adrenal vitamin C in laparotomized anaesthetized rats treated with cortisone and corticotrophin*

Results are given for individual animals, with means ± S.E. at the foot of the Table. X, Ascorbate + dehydroascorbate + dioxogulonte.

Time of collection of venous effluent (min.)	Vol. of venous effluent (ml.)	Content in venous effluent (μg.)		Wt. of adrenal (mg.)		Assessed initial content* of left adrenal (μg.)		Assessed loss† by left adrenal (μg.)		Gain‡ by venous effluent (μg.)	
		X	Ascorbate	Right	Left	X	Ascorbate	X	Ascorbate	X	Ascorbate
18	2·43	38	35	21·6	15·3	56	55	9	6	7	6
35	1·34	29	28	26·9	24·4	93	98	18	20	14	13
26	1·74	36	26	26·8	27·5	90	92	10	14	23	19
21	0·78	17	17	23·8	22·1	75	74	6	7	12	9
31	2·21	42	44	21·9	30·7	116	116	7	7	6	4
30	1·55	30	30	24·7	34·8	124	128	33	30	16	16
34	0·84	16	16	16·1	17·2	68	66	10	11	5	5
38	1·95	35	37	22·9	27·9	72	67	8	9	5	7
31	1·02	18	19	18·0	22·4	75	74	2	5	4	4
37	3·25	45	43	15·9	15·6	46	43	8	8	5	4
40	2·80	54	48	34·2	38·1	106	93	4	3	3	4
35	3·08	38	35	22·6	24·8	60	56	3	3	4	4
26	2·13	41	40	20·7	21·5	52	49	5	5	6	7
Mean ± S.E.	1·94 ± 0·22	33·8 ± 3·1	32·1 ± 2·9	22·7 ± 1·3	24·8 ± 1·8	79·5 ± 2·1	77·7 ± 2·4	9·5 ± 2·3	9·8 ± 2·1	8·4 ± 1·6	7·9 ± 1·4

* Wt. of left adrenal × initial concn. in right adrenal.
† Wt. of left adrenal × (initial concn. in right adrenal − final concn. in left adrenal).
‡ Vol. of effluent venous blood × (concn. in effluent venous blood − concn. in arterial blood).

ascorbate had been converted by the blood into ascorbate (allowance was made for ascorbate initially present in the blood and for the reducing material arising spontaneously from the added dehydroascorbate). Similar results were obtained from experiments with blood haemolysate. Thus, as Panteleeva (1950) has shown, the reducing capacity of rat blood is very small compared with that of human blood (Lloyd & Parry, 1954), and it is unlikely that the ascorbate in adrenal venous blood was formed from released dehydroascorbate.

DISCUSSION

The losses of ascorbate plus dehydroascorbate plus dioxogulonate from the adrenal in the present experiments are smaller than those reported by, for example, Slusher & Roberts (1957). Their finding that there is a correspondence between the loss by the adrenal gland and the gain by the adrenal-vein blood of these substances is confirmed by our results. The ratio of mean gain to mean loss is 0·88 for ascorbate plus dehydroascorbate plus dioxogulonate and 0·81 for ascorbate alone; the corresponding correlation coefficients are 0·54 and 0·70 respectively both being statistically significant ($P < 0·05$ and $< 0·01$ respectively). The results given in Table 1 show in every animal that although substantial amounts of ascorbate were present in the adrenal gland and in the adrenal-vein blood there was little if any dehydroascorbate or dioxogulonate. The vitamin C lost by the adrenal and gained by adrenal-vein blood in our experiments is therefore ascorbate. These conclusions are consistent with the work of Briggs & Toepel (1958) but they do not support the suggestion of Salomon (1957, 1958) that the loss of vitamin C by the adrenal gland is not in the form of ascorbate.

SUMMARY

1. Normal rats were given cortisone, and then anaesthetized, laparotomized, and given corticotrophin. The right adrenal was removed soon after laparotomy and the left adrenal was removed after a further period of about half an hour, all its venous blood having been collected by cannulation; a corresponding arterial sample was also collected.

2. Ascorbate and ascorbate plus dehydroascorbate plus dioxogulonate were estimated in parallel in the two glands and blood samples; little if any dehydroascorbate or dioxogulonate was detected in the adrenal gland or in adrenal-vein blood. The vitamin C leaving the adrenal for the blood during these experiments was thus almost entirely ascorbate.

3. Approximately 85% of the vitamin C lost by the left adrenal was detected in its venous blood.

S. L. is grateful to Professor E. G. T. Liddell, F.R.S., for laboratory facilities and to the West Bengal Government, India, for a State Scholarship. We are greatly indebted to Mr E. Aldsworth for skilled assistance.

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