The Polarographic Estimation of Steroid Hormones

6. DETERMINATION OF INDIVIDUAL ADRENOCORTICAL HORMONES IN HUMAN PERIPHERAL BLOOD

By C. J. O. R. MORRIS and D. C. WILLIAMS
Endocrine Unit, The London Hospital, London, E. 1

(*Received* 22 *October* 1952)

The first attempt at a quantitative method for the estimation of adrenocortical hormones in human peripheral blood appears to be that of Corcoran & Page (1948) who adapted their method for urinary formaldehydogenic steroids to blood. More recent studies have shown that their values were much too high, presumably due to lack of specificity in the analytical method. Several workers have investigated the adrenal venous effluent of experimental animals, particularly after stimulation with adrenocorticotrophic hormone. Vogt (1943) demonstrated the presence of these hormones in the adrenal blood of dogs by bioassay after various stimulation methods and assessed the amounts in terms of cortical extract. Nelson, Reich & Samuels (1950) obtained evidence for the presence of 17-hydroxycorticosterone in the adrenal venous blood of dogs stimulated with adrenocorticotrophic hormone, while Reich, Nelson & Zaffaroni (1950) later confirmed this by isolation. Bush (1951, 1952) fractionated blood extracts by paper chromatography, and compared the adrenal venous effluent from several mammalian species. Nelson, Samuels, Willardson & Tyler (1951) and Nelson & Samuels (1952) have developed a convenient method for 17:21-dihydroxy-20-ketosteroids in human peripheral blood, based on chromatography on magnesium silicate-Celite columns followed by estimation of the chromatographic fractions by the phenylhydrazine-sulphuric acid reaction of Porter & Silber (1950) which is believed to be specific for steroids having a 17:21-dihydroxy-20-keto grouping. Nelson *et al.* (1951) found an average normal range of 4–10 μg. 17-hydroxysteroids/100 ml. of blood. They examined their 17-hydroxysteroid fraction by paper chromatography using the method of Zaffaroni, Burton & Keutmann (1950) and concluded that it contained only 17-hydroxycorticosterone.

The present work has involved application of the methods of partition chromatography and polarographic estimation of Δ^4-3-ketosteroids developed in this laboratory (Butt, Morris, Morris & Williams, 1951) for the estimation of progesterone in blood. A method has been worked out for the determination of corticosterone, dehydrocorticosterone, 17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone in human peripheral blood. Some preliminary results have been communicated earlier (Morris & Williams, 1953).

METHODS

Preliminary extraction and fractionation. All solvents used in this work have been redistilled using a 30 cm. fractionating column packed with Fenske spirals. Satisfactory results can only be obtained if a control experiment using 20 ml. of water instead of plasma yields a negligible polarographic blank with all solvents and reagents used. All vacuum evaporations are carried out below 50°.

Approximately 50 ml. of blood are collected using heparin as anticoagulant. The plasma is separated as soon as possible after collection, 20 ml. of plasma being used for the estimation. The proteins are precipitated by the gradual addition of ethanol (60 ml.) with vigorous stirring. The mixture is thoroughly shaken and centrifuged. The supernatant liquid is separated and the precipitate washed by vigorous stirring with a further 20 ml. of ethanol. After centrifugation, the wash liquid is combined with the first extract and evaporated to dryness under reduced pressure at 30–40°. The solid residue is dissolved in 20% (v/v) ethanol (20 ml.) and extracted three times with 20 ml. portions of ethyl acetate. The ethyl acetate extracts are combined and evaporated to dryness under reduced pressure. The residue is transferred quantitatively to a 5 ml. centrifuge tube using 2 ml. of 20% (v/v) ethanol and warming to 30–40° if necessary to facilitate solution. The ethanol solution is stored at −10° overnight and rapidly centrifuged while still cold. The insoluble residue is washed with ice-cold 20% ethanol (0·5 ml.) and again centrifuged. Extract and washings are combined and the solvent removed under reduced pressure. The residue is dissolved in 50% (v/v) ethanol (10 ml.) and extracted with CCl_4 (10 ml.). The alcoholic phase is separated and the solvent removed under reduced pressure.

A reverse-phase partition column (Howard & Martin, 1950), is set up using Hyflo Super-cel (4 g.) which had been previously treated with dichlordialkylsilane (Teddol, British Thomson-Houston, Rugby) as supporting phase. The stationary phase is light petroleum (b.p. 80–100°; 2 ml./4 g. Super-cel). 50% (v/v) ethanol which has been thoroughly equilibrated with light petroleum is used as mobile phase. The treated Super-cel is mixed with the stationary phase and mobile phase added to make a slurry. A glass tube 1 cm. in diam. is packed by the method described by Butt *et al.* (1951), to form a column about 4 cm. long. The residue from the CCl_4 extraction is taken up in

50% (v/v) ethanol (0·5 ml., equilibrated with light petroleum), and introduced on to the column. Pressure (3 cm. Hg) is applied, and after the first 0·5 ml. has been forced into the column, the process is repeated by washing the flask with a further 0·5 ml. of 50% (v/v) ethanol and transferring it to the column. 3 ml. of equilibrated 50% ethanol are now added to the column and pressure applied. The total corticosteroids are contained in the first 4 ml. of eluate, while remaining lipids are extracted into the light petroleum. The alcoholic phase is evaporated to dryness *in vacuo*.

Chromatographic separation of corticosteroids. The extract prepared in the manner described contains the corticosteroids and is practically free from fats and phospholipins.

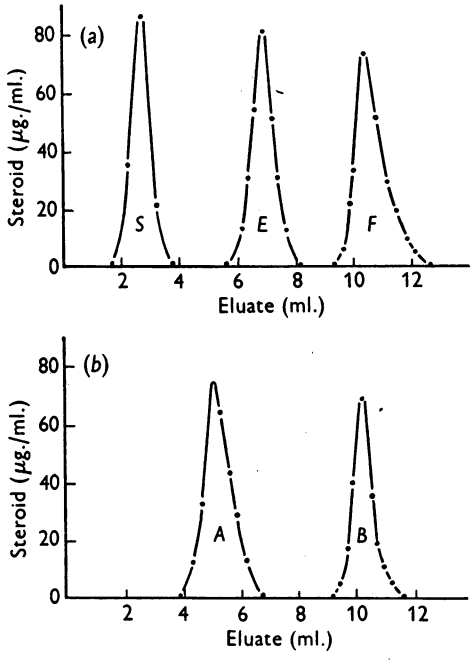

Fig. 1. Chromatographic separation of corticosteroids. (*a*) System toluene-25% ethanol-water. (*b*) System 20% light petroleum-toluene-ethylene glycol. *S*, 11-deoxy-17-hydroxycorticosterone; *E*, cortisone; *F*, 17-hydroxycorticosterone; *A*, 11-dehydrocorticosterone; *B*, corticosterone.

The individual corticosteroids are separated by means of chromatography on two partition columns. The first column uses 25% (v/v) aqueous ethanol as stationary phase and toluene as mobile phase. The two phases must be thoroughly equilibrated before use. Hyflo Super-cel (3 g.) is thoroughly mixed with 2 ml. of stationary phase. The mixture is then made into a slurry with excess mobile phase and packed as described by Butt *et al.* (1951) to form a column 8 × 0·6 cm. Such columns should run under an 8–10 cm. head of mobile phase at about 0·8 ml./hr. Excess mobile phase is removed from the top of the column and the liquid level forced down under 1–2 cm. Hg positive pressure until it is about 1 mm. below the surface of the column. Sufficient dry Super-cel is now added to form a layer 2–3 mm. deep on top of the column which is packed down well. The blood extract dissolved in 0·1 ml. stationary phase is carefully added to the dry Super-cel with a capillary pipette and forced down under positive pressure (1–2 cm. Hg). Mobile phase is added with a capillary pipette so that the surface of the column is not disturbed. A solvent reservoir is attached and the column run under gravity (8–10 cm. head of toluene) overnight. 1 ml. fractions are collected by means of an automatic fraction collector. The first 4 ml. of effluent contains 11-dehydrocorticosterone, corticosterone and 11-deoxy-17-hydroxycorticosterone, which are not resolved. 11-Dehydro-17-hydroxycorticosterone (cortisone, compound E) is collected in fractions 7–8 and 17-hydroxycorticosterone (compound F) in fractions 10–11. The first 4 ml. of effluent are freed of solvent under reduced pressure and dissolved in 0·1 ml. of toluene for transference to the second column. In this column ethylene glycol is the stationary phase and 20% (v/v) light petroleum (b.p. 80–100°)—toluene the mobile phase. Both phases are thoroughly equilibrated before use. The technique of packing and applying the material to be separated is identical with that described above for the aqueous ethanol-toluene column. The second column should also be 8 × 0·6 cm. and run at 0·8 ml./hr. 11-Dehydrocorticosterone separates in fractions 4–5 and corticosterone in fractions 11–12. 17-Hydroxy-11-deoxycorticosterone (if present) is recovered in fractions 17–23.

The course of the separations is shown in Fig. 1.

Solvent is removed from the fractions under diminished pressure and the residues converted into the Girard hydrazones for polarography.

Polarographic estimations

Reagents

tert.-*Butanol.* The commercial solvent is purified by distillation through a 30 cm. column packed with Fenske spirals. The redistilled solvent is shaken ten times successively with an equal volume of 18% (w/v) aqueous KCl solution ('Polaritan' KCl, Hopkins & Williams Ltd.), the aqueous phase being discarded after each extraction. The *tert.*-butanol phase is dried first over anhydrous Na_2SO_4 and then over metallic Na. It is finally redistilled. *tert.*-Butanol purified in this way should give no polarographic wave under the conditions described below and is stable for at least several months.

Girard T reagent. The commercial reagent is purified by dissolving in a minimal quantity of hot ethanol and the solution heated with charcoal under a reflux condenser for 1 hr. The charcoal is removed by filtration while the solution is hot and the Girard reagent allowed to crystallize. It is recrystallized a further three times from ethanol.

Acetic acid. Anhydrous acetic acid is prepared from acetic anhydride by the method of Wolfe, Hershberg & Fieser (1940).

tert.-*Butanol-acetic acid-Girard T reagent.* This is made up immediately before use by adding 0·04 ml. of a 10% (w/v) solution of Girard T reagent in anhydrous acetic acid to 2 ml. of purified anhydrous *tert.*-butanol.

Polarographic base solution. All salts used are 'Polaritan' reagents (Hopkins and Williams Ltd.). The base solution contains 0·5N-KCl solution, 0·2N-KOH solution and water (1:2:1, v/v), adjusted to pH 5·6 with glacial acetic acid. It is made up before use from the stock solutions, which appear to be stable indefinitely.

Method

The solvent-free residues from chromatography are stored overnight *in vacuo* over P_2O_5. 0·05 ml. of the *tert.*-butanol-acetic acid-Girard T reagent is added to each tube, the tube stoppered and heated for exactly 2 min. at 100°. The butanol-acetic acid mixture is removed under reduced pressure below 50°. Each fraction is treated with 0·2 ml. of polarographic base solution and 0·2 ml. of toluene. The tubes are shaken, allowed to settle and the aqueous layers transferred to the polarographic cell (Fig. 2). The cell shown

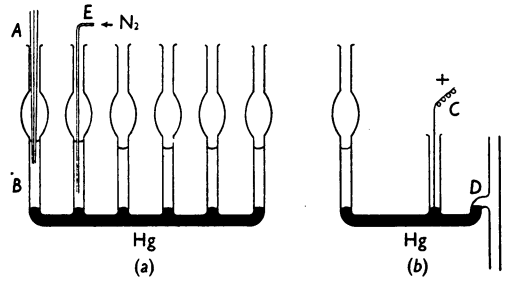

Fig. 2. Micropolarographic cell. (*a*) front, (*b*) side; *A*, dropping Hg electrode; *B*, solution; *C*, Pt anode contact; *D*, Hg overflow; *E*, polythene capillary inlet for N_2 to remove O_2.

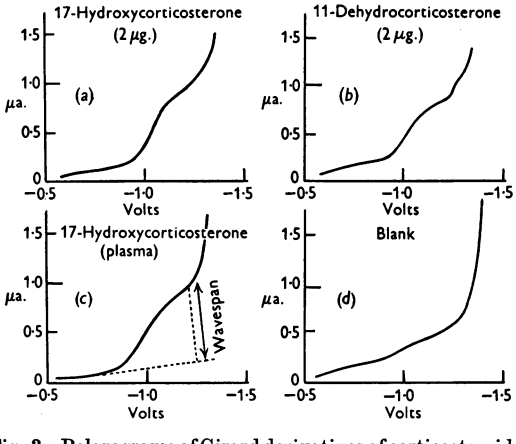

Fig. 3. Polarograms of Girard derivatives of corticosteroids. Sensitivity: full scale = 2 μa. (*a*) 17-Hydroxycorticosterone. (*b*) 11-Dehydrocorticosterone. (*c*) 17-Hydroxycorticosterone from human-plasma. (*d*) Blank 'wavespan' 2 mm.

enables six samples to be analysed in rapid succession, O_2 in the test solution is removed by bubbling N_2 through for 30–60 sec. Recording and evaluation of polarograms is carried out as described earlier (Barnett, Henly & Morris, 1946), the wavespan of the Δ^4-3-ketosteroid wave being measured. The amounts of steroid are estimated from standards for each individual component, polarographed at the time of estimation. Typical polarograms of 17-hydroxycorticosterone and 11-dehydrocorticosterone are shown in Fig. 3 (*a*) and (*b*).

It will be seen that whereas 11-dehydrocorticosterone gives two polarographic waves characteristic of the Δ^4-3-keto group and of the 20-keto group, 17-hydroxycorticosterone gives only the Δ^4-3-keto wave. All 17-hydroxycorticosteroids examined, 17-hydroxycorticosterone (three specimens), 11-dehydro-17-hydroxycorticosterone (two free alcohol and one acetate specimens) and 11-deoxy-17-hydroxycorticosterone show only the Δ^4-3-keto wave. On the other hand, corticosterone, 11-dehydrocorticosterone and 11-deoxycorticosterone all show both waves. Half-wave potentials of the Girard derivatives of these steroids measured against the saturated calomel electrode at 25° are given in Table 1.

Table 1. *Half-wave potentials of Girard T derivatives of corticosteroids*

Compound	Δ^4-3-Keto wave (V.)	20-Keto wave (V.)
11-Dehydrocorticosterone	−1·08	−1·36
Corticosterone	−1·09	−1·37
11-Deoxycorticosterone	−1·11	−1·35
11-Deoxy-17-hydroxycorticosterone	−1·15	—
11-Dehydro-17-hydroxycorticosterone (cortisone)	−1·11	—
17-Hydroxycorticosterone	−1·12	—

Wolfe *et al.* (1940) report double waves for 11-deoxycorticosterone acetate, corticosterone and 11-dehydro-17-hydroxycorticosterone. The discrepancy in the last case is difficult to explain, as repetition of the experiments under the conditions of Wolfe *et al.* has never yielded a 20-keto wave with any 17-hydroxycorticosteroid examined. It may be noted that Wolfe *et al.* give 208° for the melting point of their specimen of 11-dehydro-17-hydroxycorticosterone, whereas 215° is now generally accepted. A typical wave given by 17-hydroxycorticosterone from plasma is shown in Fig. 3 (*c*). The method of measuring the 'wavespan' (Wolfe *et al.* 1940) is also shown. 2 μg. of 17-hydroxycorticosterone gives a wavespan of 26 mm., while 2 μg. of 11-dehydrocorticosterone gives a wavespan of 21 mm., measured at sensitivity 2 μa. = full scale of the Tinsley polarograph with a dropping Hg electrode of characteristics, $t = 2{\cdot}30$ sec., $m = 56{\cdot}3$ mg. Since the wavespan can be measured with an accuracy of ±1 mm. allowing for an uncertainty of this magnitude in the blank, the method should have an accuracy of ±10% even at the lower levels encountered in normal human plasma. Fig. 3 (*d*) shows a blank obtained after extracting 20 ml. of water by the whole procedure described above.

RESULTS

Recoveries of the five corticosteroids investigated, added to 20 ml. of plasma, are given in Table 2.

Under these conditions, recovery is in most cases better than 80% at the 5 and 10 μg. levels. The recoveries of the individual steroids also indicate that there is no interconversion during processing.

Table 3 gives values for the corticosteroids in normal subjects aged 20–45 years. In no case up to the present has 11-deoxy-17-hydroxycorticosterone been detected.

Table 2. *Recovery of corticosteroids added to plasma*

Compound	Initial value (μg.)	Added (μg.)	Found (μg.) (1)	Found (μg.) (2)	Found (μg.) (3)	Recovery (%) (1)	Recovery (%) (2)	Recovery (%) (3)
11-Dehydrocorticosterone	0·9	5·0	5·0	5·1	5·3	82	84	88
	0·9	10·0	9·4	9·3	—	85	84	—
	0·7	10·0	9·2	—	—	85	—	—
Corticosterone	1·7	5·0	5·9	5·7	5·8	84	80	82
	1·7	10·0	10·3	9·7	—	86	80	—
	1·5	10·0	9·3	—	—	76	—	—
11-Deoxy-17-hydroxycorticosterone	0·0	10·0	7·1	7·6	7·3	71	76	73
Cortisone	0·8	5·0	5·1	5·3	4·8	86	90	80
	0·8	10·0	10·0	9·8	—	92	90	—
	0·7	10·0	9·4	—	—	86	—	—
17-Hydroxycorticosterone	1·4	5·0	5·4	5·5	5·4	80	82	80
	1·4	10·0	10·1	10·4	—	87	90	—
	2·1	10·0	11·2	—	—	91	—	—

Table 3. *Blood corticosteroids in normal subjects and in pregnancy*

No.	Sex	Compound (μg./100 ml. plasma) 11-Dehydro-corticosterone	Corticosterone	Cortisone	17-Hydroxy-corticosterone
1	M.	4·5	8·5	4·0	7·0
2	M.	3·5	7·5	3·5	10·5
3	F.	5·0	10·5	4·0	7·5
4	F.	2·0	4·5	2·5	6·5
5	F.	3·0	9·0	4·5	9·0
6	M.	3·0	4·0	4·0	8·5
7	M.	4·0	10·5	5·0	10·0
	Pregnancy (term, weeks)				
1	Full	5·0	18·0	7·5	18·5
2	34	8·5	15·0	9·0	23·5
3	32	4·5	12·5	4·5	15·5
4	34	2·5	7·0	6·5	8·5

Table 4. *Blood corticosteroids in normal and adrenocorticotropin (ACTH)-treated males*

Case	Treatment	Compound (μg./100 ml. plasma) 11-Dehydro-corticosterone	Corticosterone	Cortisone	17-Hydroxy-corticosterone
1	None	2·0	5·5	2·5	5·0
	25 i.u. ACTH	3·0	19·5	8·5	20·5
2	None	5·0	10·0	4·5	8·0
	25 i.u. ACTH	9·0	18·5	7·5	20·0
3	None	3·5	9·0	5·0	9·0
	25 i.u. ACTH	10·5	16·5	7·5	16·0
4 (Resting)	None	7·5	10·0	5·0	12·5
	25 i.u. ACTH	14·5	19·0	40·0	56·5
5 (Resting)	None	4·0	7·5	5·0	8·0
	25 i.u. ACTH	17·5	27·0	33·5	42·5
6 (Resting)	None	2·5	6·5	3·0	10·0
	25 i.u. ACTH	22·0	45·5	44·5	75·0

In one case the concentrations of the corticosteroids in blood and plasma were compared using the same blood for both sets of determinations. The blood was haemolysed by shaking with water and subsequently analysed as described for plasma. The blood used had a haematocrit value of 46%. Accordingly, the corticosteroid values (μg.) from 20 ml. of plasma were compared with those from 37 ml. of blood. The values found were: 11-dehydrocorticosterone; plasma 0·8, blood 1·0: corticosterone; plasma 2·1, blood 2·4: cortisone; plasma 1·0, blood 0·8: 17-hydroxycorticosterone, plasma 2·0, blood 1·8. It is thus evident that, in agreement with the findings of Nelson *et al.* (1951), the corticosteroids are present solely in the plasma and that no significant loss occurs during separation of the plasma.

Table 3 also gives values for the corticosteroids in normal pregnancies of varying duration. In accordance with the findings of other workers on urinary corticosteroids, the blood levels, particularly of corticosterone and 17-hydroxycorticosterone, appear to be elevated in pregnancy.

Table 4 illustrates the effect of pituitary adrenocorticotrophic hormone (Acthar, Armour and Co.) on blood corticosteroid levels. A blood sample was withdrawn for control determinations, 25 i.u. of adrenocorticotrophic hormone injected intramuscularly and a further blood sample taken 1·5 hr. later. In all cases an increase in the blood levels of all four corticosteroids was observed, the increase in 17-hydroxycorticosterone being the most marked.

DISCUSSION

The presence in peripheral blood of 11-dehydrocorticosterone and 11-dehydro-17-hydroxycorticosterone as well as the corresponding 11-hydroxysteroids, which had already been observed in adrenal venous blood, was somewhat surprising, especially as Nelson & Samuels (1952) identified 17-hydroxycorticosterone as the only steroid present in their 17-hydroxy fraction from human peripheral blood. It is however possible that their extraction method did not recover 11-dehydro-17-hydroxycorticosterone quantitatively from blood and that the reduced amounts present escaped detection during paper chromatography, especially as, in our experience, the 11-dehydro-17-hydroxycorticosterone level is invariably lower than (usually less than half) the 17-hydroxycorticosterone level.

In view of the small quantities available, a rigorous identification of the substance running in the 11-dehydro-17-hydroxycorticosterone position was not possible. However the following evidence is available as to its identity: (1) The substance has the same partition coefficient as 11-dehydro-17-hydroxycorticosterone in the system toluene—25% ethanol. (2) The substance is a Δ^4-3-ketosteroid. It does not show a 20-keto wave on polarography of the Girard hydrazone indicating that it is probably a 17-hydroxysteroid. (3) It reduces blue tetrazolium salt in ethanolic potassium hydroxide solution, indicating the presence of a ketol side chain Chen & Tewell (1951). (4) It shows the yellow fluorescence in ultraviolet light after heating with alkali on filter paper, suggested by Bush (1952) as characteristic of an $\alpha\beta$-unsaturated ketonic group in a reduced naphthalene ring system. (5) It gives a positive Porter-Silber reaction with phenylhydrazine and sulphuric acid, which was found by the authors to be characteristic of 17:21-dihydroxy-20-ketosteroids.

In view of this evidence the substance is tentatively identified as 11-dehydro-17-hydroxycorticosterone although more positive identification must await the accumulation of larger quantities. Similarly the compound running in the 11-dehydrocorticosterone positions has been identified as a Δ^4-3:20-ketosteroid with a reducing side chain.

It is possible that the 11-ketosteroids arise from the 11-hydroxysteroids as a result of tissue oxidation and that the former are not present as such in the adrenal venous effluent.

The method described can give results of value particularly in cases of adrenal dysfunction; other analytical methods such as the Porter-Silber reaction and the blue tetrazolium reduction method of Mader & Buck (1952) could also be applied to the chromatographic fractions. Intercomparison of such methods might extend our knowledge of the metabolism of the corticosteroids in the body.

SUMMARY

1. The separation of adrenocortical steroids by partition chromatography has been applied to blood extracts and combined with a micro-polarographic technique to give a method for the estimation of these substances in human peripheral blood.

2. Analytical values for the compounds corticosterone, 11-dehydrocorticosterone, 11-dehydro-17-hydroxycorticosterone and 17-hydroxycorticosterone present in normal and pregnancy bloods are given.

3. The effect of pituitary adrenocorticotrophic hormone on the blood levels of these steroids in normal subjects has been investigated.

The authors are indebted to Prof. E. C. Kendall for a gift of 11-dehydrocorticosterone and to Dr W. J. Haines for gifts of corticosterone and 17-hydroxycorticosterone.

REFERENCES

Barnett, J., Henly, A. A. & Morris, C. J. O. R. (1946). *Biochem. J.* **40**, 445.
Bush, I. E. (1951). *J. Endocrin.* **7**, lxxxii.
Bush, I. E. (1952). *Biochem. J.* **50**, 370.
Butt, W. R., Morris, P., Morris, C. J. O. R. & Williams, D. C. (1951). *Biochem. J.* **49**, 434.
Chen, C. & Tewell, H. E. (1951). *Fed. Proc.* **10**, 377.
Corcoran, A. C. & Page, I. H. (1948). *J. Lab. clin. Med.* **33**, 1326.
Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.
Mader, W. J. & Buck, R. R. (1952). *Analyt. Chem.* **24**, 666.
Morris, C. J. O. R. & Williams, D. C. (1953). *Ciba Foundation Colloquia on Endocrinology*, **7** (in the Press).
Nelson, D. H., Reich, H. & Samuels, L. T. (1950). *Science*, **111**, 578.
Nelson, D. H. & Samuels, L. T. (1952). *J. clin. Endocrin.* **12**, 519.
Nelson, D. H., Samuels, L. T., Willardson, D. G. & Tyler, F. H. (1951). *J. clin. Endocrin.* **11**, 1021.
Porter, C. C. & Silber, R. H. (1950). *J. biol. Chem.* **185**, 201.
Reich, H., Nelson, D. H. & Zaffaroni, A. (1950). *J. biol. Chem.* **187**, 411.
Vogt, M. (1943). *J. Physiol.* **102**, 341.
Wolfe, J. K., Hershberg, E. B. & Fieser, L. F. (1940). *J. biol. Chem.* **136**, 653.
Zaffaroni, A., Burton, R. B. & Keutmann, E. H. (1950). *Science*, **111**, 2871.

Lipovitellin

3. THE EFFECT OF SUGARS ON THE STABILITY OF FREEZE-DRIED LIPOVITELLIN

By J. C. HAWKE and C. H. LEA
Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge, and Department of Scientific and Industrial Research

(*Received* 20 *October* 1952)

The effects of freezing and drying lipovitellin and of storage in the frozen and dried states have already been investigated (Lea & Hawke, 1952*a–c*). The present paper describes attempts to control the deteriorative changes which occur during and after freeze-drying by the addition of carbohydrate before freezing.

The value of sugars, particularly lactose, for the stabilization of proteins during drying has previously been reported (Brosteaux & Eriksson-Quensel, 1935). Sucrose and glycerol have been shown to inhibit the heat coagulation of rabbit serum and of egg albumin (Beilinsson, 1929), and various hexoses and pentoses, including glucose, fructose, mannose, arabinose and xylose, have been found to protect proteins from heat denaturation in aqueous solution as measured by the liberation of SH groups (Ball, Hardt & Duddles, 1943). Moran (1925) found that added sugars prevented the increase in viscosity which normally occurs in egg yolk during freezing and thawing, and sucrose and glycerol are used commercially for this purpose. The presence of added carbohydrate, especially lactose or in less degree sucrose, has been found to retard loss of solubility and loss of aerating power of spray-dried egg during storage, but glucose and particularly arabinose accelerated these changes (Brooks & Hawthorne, 1943). On the other hand, Fry & Greaves (1951) found that glucose, lactose, sucrose, raffinose and xylose all increased the survival of bacteria during freeze-drying and subsequent storage.

For the present purpose sucrose, lactose and glucose have been selected as representative sugars, and their effects on the solubility and free lipid content of lipovitellin have been determined after freeze-drying and during subsequent storage in the 'dry' state under various conditions.

METHODS

A lipovitellin suspension containing approximately 10% solids was prepared as previously described (Lea & Hawke, 1951, 1952*c*), adjusted to the required pH, allowed to stand at 0° overnight in the presence of sugar equal in weight to the lipoprotein present, mixed, and frozen rapidly in shallow layers in thin metal trays by immersion in liquid at −60 to −70°. After reducing the moisture content of the frozen material to about 2% by freeze-drying for 2 days in the presence of P_2O_5 (Lea & Hawke, 1952*c*) the product was mixed by brief agitation in a blender at 0° and the fluffy, off-white powder stored at 37° over H_2SO_4 solutions producing the required water vapour pressures.

Lipovitellin and samples containing respectively sucrose, lactose and glucose were prepared at pH 6·8, and stored at 30, 70 and 92% relative humidity (R.H.). At pH 5·2 lipovitellin and lipovitellin-sucrose only were prepared and storage was at 70% R.H. only.