- Mann, T. & Lutwak-Mann, C. (1948). *Biochem. J.* **43**, 266.
 Needham, J. (1924). *Biochem. J.* **18**, 891.
 Nixon, D. A. (1952). M.Sc. Thesis: London University.
 Nixon, D. A. (1955). *J. Physiol.* **129**, 272.
 Northam, B. E. & Norris, F. W. (1952). *J. gen. Microbiol.* **7**, 245.
 Posternak, S. & Posternak, T. (1929). *Helv. chim. acta*, **12**, 1165.
 Posternak, T., Schopfer, W. H. & Reymond, D. (1955). *Helv. chim. acta*, **38**, 1283.
 Scherstén, B. (1936). *Skand. Arch. Physiol.* **74**, suppl. 7.
 Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.
 Somogyi, M. (1945). *J. biol. Chem.* **160**, 69.
 Wallenfels, K. (1951). *Naturwissenschaften*, **38**, 238.
 Walton, A. (1945). *Notes on Artificial Insemination of Sheep, Cattle and Horses*. London: Holborn Surgical Instrument Co.
 Woolley, D. W. (1940). *J. biol. Chem.* **136**, 113.
 Woolley, D. W. (1941a). *J. biol. Chem.* **140**, 461.
 Woolley, D. W. (1941b). *J. biol. Chem.* **139**, 29.
 Woolley, D. W. (1942). *J. exp. Med.* **75**, 277.

Tissue Components of the Domestic Fowl

1. THE D-GLUCOSE CONTENT OF WHOLE BLOOD

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Microanalyses of reducing sugars in avian bloods have been attempted many times with the general indication that the concentrations are markedly higher than those found in mammals. Most previous workers have used methods that are affected by *restreduktion* [non-carbohydrate blood components (cf. Herbert & Bourne, 1930)]. In a few instances 'fermentable sugar' has been estimated (Cassidy, Dworkin & Finney, 1926; Erlenbach, 1938; Fujita & Iwataki, 1931; Cavett, 1939). However, the addition of a washed yeast suspension to deproteinized blood, in which only few of the constituents have ever been identified, need not necessarily result in absorption of reducing sugars alone; other reducing components may be taken up by the yeast. Indeed, during fermentation, reducing substances can be formed [cf. Meyer (1913) and Merten (1938)].

The Folin & Wu (1919) method, as applied to the analysis of blood sugar in fowls, has been adversely criticized by Cassidy *et al.* (1926), Gulland & Peters (1930) and Fujita & Iwataki (1931). Ferricyanide methods, mainly that of Hagedorn & Jensen (1923), have also received adverse criticism by Gulland & Peters (1930), Erlenbach (1938) and Fujita & Iwataki (1931).

Few determinations of the blood sugar of fowls by titrimetric copper methods have been recorded. Strange, Dark & Ness (1955) have shown that both ferricyanide and copper reagents can be interfered with by amino acids as well as by creatine, uric acid, etc. and that Cd^{2+} , Ba^{2+} and Hg^{2+} will not satisfactorily remove these substances. These authors, likewise, found the copper reagents superior to ferricyanide.

Table 1 summarizes those papers known to the author where sufficient data have been given to allow comparison with the results of this paper. It is not claimed that this table is complete; because of indexing lacunae this field is difficult to search. The breeds of fowls, where known, are not stated in the table; there is no evidence so far of any influence of this factor on the blood sugar. It was therefore considered necessary, as a preliminary to future experimental work, that a search be made for a simple method which would determine the blood glucose in agreement with assays using D-glucose oxidase. The stabilized benzidine reagent of Jones & Pridham (1954) was found not to be affected by non-sugar components of three kinds of blood-'filtrates'; it gives results agreeing closely with blood-glucose determinations by means of D-glucose oxidase. The author is grateful to Professor A. St George Huggett, who drew his attention to the possibilities of the benzidine method (cf. Huggett, 1956).

METHODS

The birds used were Brown Leghorns taken either from pure lines or crosses from these lines, and were superficially healthy. No genetical influence on the blood-sugar levels was found. Unless fasted, the birds were allowed free access to the normal food used at this Centre.

This is a mash consisting of (in parts by weight) parings, 15.5; bran, 14.5; maize meal, 22.0; Sussex ground oats, 20.0; white fish meal, 9.5; dried unextracted brewer's yeast, 4.0; dried skimmed milk, 5.0; dried grass meal, 5.0; ground limestone, 1.0; common salt, 0.5; cod-liver oil (stabilized), 2.0; mineral mixture, 1.0. The last-named has the following composition (lb./approx. ton): exsiccated iron sulphate, 56; hydrated manganese sulphate, 124; limestone flour, 1399; sterilized feeding bone flour, 314; common salt, 336;

potassium iodide, 1½; cobalt sulphate, 1½; copper sulphate, 34. Mash is fed in roughly equal amounts with a grain mixture, consisting of kibbled maize, 33 lb.; whole wheat, 33 lb.; whole oat groats, 34 lb. The birds have *ad libitum* supplies of a mixture of flint grit and oyster-shell grit.

Blood sampling. (a) For large volumes, the animals were anaesthetized with veterinary Nembutal (45–60 mg.) injected into the alar vein. When anaesthesia was complete the abdomen was opened and blood collected directly from the heart. (b) Amounts of 1–5 ml. were taken by syringe from the alar vein. (c) As anticoagulants, citrate, heparin or sodium laminarin sulphate (O'Neill, 1955; O'Neill & Hawkins, 1955) were tried. Citrate (about 2 mg./ml.) was found to be satisfactory.

'Deproteinized' blood filtrates. Type A. Blood (1 vol.) was added to water (9 vol.) containing 0.24 mg. of acetic acid for each millilitre of blood (cf. Hunter, 1956). After mixing and laking, the whole was kept in a boiling-water bath, with frequent shaking, until coagulation was complete (5 min.), and filtered (Whatman no. 30 paper). Unless these filtrates were used immediately they were saturated with benzoic acid and stored at –20°.

Type B. The procedures of Somogyi (1945), with ZnSO₄ and Ba(OH)₂ in the cold, and of Fujita & Iwataki (1931), with CdSO₄ and NaOH in the hot, were followed exactly.

Type C. Blood (1 vol.) was added to 10% (w/v) trichloroacetic acid solution (9 vol.), and the mixture was shaken,

kept for about ½ hr., and filtered through a Whatman no. 30 paper. This deproteinization is most convenient for routine work.

Detection of D-glucose. Paper chromatography of 80% ethanolic extracts of serum and paper electrophoresis in borate buffer of concentrated (A) filtrates showed no sugar other than glucose. 1:2:5:6-Di-O-isopropylidene D-glucosufuranose (m.p. 110°), [α]_D –12° (CHCl₃), was isolated by Bell's (1947) method from type A filtrates.

Measurements of reducing power. Titrimetric determinations were made on filtrates of types A and B by the 2 ml. scale copper method of Somogyi (1952); 0.0025N-thio-sulphate was satisfactory for the titrations.

Filtrates of types A and C were examined by the benzidine colorimetric method for aldoses (Jones & Pridham, 1954); with type C filtrates it was necessary for the appropriate amounts of trichloroacetic acid to be present in both blanks and standards, as the colours of water and trichloroacetic acid solutions are not spectrophotometrically comparable. Duplicate determinations invariably agreed so closely that single measurements are sufficient for routine analyses. With some bloods a slight precipitate, easily removed on the centrifuge, formed on heating with the benzidine reagent.

With both methods, standard glucose solutions prepared and stored as described by Bacon & Bell (1948) were invariably run concurrently with each set of analyses.

Table 1. *Summary of published values for the 'blood sugar' (mg./100 ml. of whole blood) in fowls*

Methods used. Type I: the Folin-Wu type (Folin & Wu, 1919; Folin, 1929; Folin & Malmros, 1929; Benedict, 1925; Rothberg & Evans, 1923). Type II: the Hagedorn-Jensen type (Hagedorn & Jensen, 1923; Fujita & Iwataki, 1931; Burr & Tanimoto, 1950; Kingsley & Reinhold, 1949). The last two are colorimetric. Type III: miscellaneous (Baudoin & Lewin, 1927; Somogyi, 1930, 1945; Shaffer & Hartman, 1921; Bertrand modified by Best, Hoet & Marks, 1926).

Sex	Condition	Methods (type)	Average values (range)	Individual values (range)	Authors
F.	Fed adult	I	139–279	91–333	1–9
		II	183–246	175–307	10–14
		III	197	175–225	16
			173	—	26
F.	Fasted adult	I	186 (155*)	—	3
		II	152–182	150–290	13, 14, 15
		III	162	148–201	16
M.	Fed adult	I	161–203 (170*)	148–260	3, 16, 17
		II	188–218	143–236	12, 14, 18, 19
M.	Fasted adult	I	180 (150*)	178–181	3
		II	172–180	78–232	18, 20
Mixed	Fed, 1–4 months	I	188†	—	25
		III	162–228	146–278	21–24
Mixed	Fasted, 1–4 months	I	148–169	—	25
		III	182–195	175–206	22, 23

* 'Fermentable reduction'.

† 424 birds, average increased with age.

Authors

(1) Scheunert & Pelchriz (1923)	(10) Schwartz & Heinrich (1928)	(19) Burrows, Fritz & Titus (1935)
(2) Thompson & Carr (1923)	(11) Fukita & Iwataki (1931)	(20) Henry, Magee & Reid (1934)
(3) Cassidy <i>et al.</i> (1926)	(12) Erlenbach (1938)	(21) Hogan, Shrewsbury & Kempton (1928)
(4) Hayden (1929)	(13) Murray & Rosenberg (1953)	(22) Corkill (1930)
(5) Horvath (1930 <i>a, b</i>)	(14) Sturkie (1955)	(23) Golden & Long (1942)
(6) Hayden & Sampson (1931)	(15) Macowan & Magee (1932)	(24) Hsu & Combs (1952 <i>a, b</i>)
(7) Dyer & Roe (1934)	(16) Rogement (1930)	(25) Opdyke (1942)
(8) Russell & Weber (1934)	(17) Gonzago (1935)	(26) Tapper & Kare (1956)
(9) Bronkhorst & Hall (1935)	(18) Krasnianski & Dsikowski (1931)	

Determination of D-glucose by D-glucose oxidase (cf. Keilin & Hartree (1948) and Bell & Manners (1952)). Samples of five large-scale type A filtrates were concentrated under reduced pressure (bath temp. below 30°) to volumes corresponding to those of the original bloods. Portions (1 ml.) were incubated in Warburg manometer flasks with catalase (1 ml.) [300 mg. of commercial beef enzyme (L. Light and Co. Ltd.) in 15 ml. of M/30 phosphate buffer, pH 6.5] and glucose oxidase solution (0.2 ml. initially in side bulb). The very large amount of crude catalase was required to produce a maximal oxygen uptake; the reason for this is unknown. The glucose oxidase was commercial material (Takamine Laboratories Inc.); 15 mg. was suspended in 10 ml. of M/30 phosphate buffer (pH 6.5), solid removed on the centrifuge and the clear, supernatant fluid stored at approx. 2°. Controls were run against the catalase alone, and against 2 mg. amounts of D-glucose. The oxygen uptakes observed in three experiments were between 96.5 and 98.9 % of the theoretical 124.4 μ l.

De-ionization of deproteinized filtrates (type A). As found by Strange *et al.* (1955), Amberlites IR-4B(OH) and IR-120(H) in equal weights did not remove glucose from solution. Experiments showed that shaking 1 vol. of a 1:10 filtrate with one-twentieth of its weight of each resin for 1 hr. removed practically all the non-protein nitrogen and the unknown substances which interfered with cuprimetric determination of the sugar.

Non-protein nitrogen. The procedure of Chibnall, Rees & Williams (1943) was followed.

RESULTS

Preliminary examination of methods. Numerous experiments on blood filtrates of types A and B indicated that the cuprimetric method was not

satisfactory. It certainly could not be applied to type A filtrates since the results, unexpectedly, were often very low (cf. Strange *et al.* 1955); the Somogyi and Fujita-Iwataki deproteinization methods gave filtrates containing more 'glucose' than could be found by D-glucose oxidase. On the other hand, the results of the benzidine colorimetric method were consistently in agreement with the true glucose, both when used directly on type A filtrates, or after deionization. The cuprimetric method used on deionized type A filtrates also gave satisfactory agreement. Despite the numerous possible sources of manipulative error, maximum differences of approximately 5 % between the glucose oxidase results and those of the benzidine method were the highest observed. It was therefore concluded that this latter easy and rapid technique was the method of choice for the subsequent survey of blood-sugar values. The blank is colourless and the reaction colour is stable for over 24 hr. Table 2 summarizes one of a number of preliminary experiments where the non-protein nitrogen of the various blood filtrates was examined as well as the apparent sugar contents.

To check that trichloroacetic acid (type C filtrates) did not affect the benzidine determinations, two sets of parallel measurements were done on types A and C filtrates made from the same blood samples. The results are given in Table 3; it will be seen that type C filtrates are satisfactory for the determination of glucose by the benzidine method.

Table 2. *Comparison of copper and benzidine methods with D-glucose oxidase method*

Three different procedures for deproteinization, and resin deionization, were employed. Glucose and non-protein nitrogen (N.P.N.) were determined as mg./100 ml. of whole blood (mean of duplicate and triplicate determinations), on five cocks.

Type A filtrates					
D-Glucose oxidase determination	275	174	179	230	173
Benzidine determination	264	169	179	212	177
Somogyi determination	160	75	85	175	80
N.P.N.	51.8	52.6	69.5	64.3	47.0
Type A filtrates, deionized					
Benzidine determination	260	169	178	218	184
Somogyi determination	253	172	176	219	186
N.P.N.	3.9	3.2	1.5	5.9	0
Somogyi filtrate					
Somogyi determination	319	209	224	274	195
N.P.N.	24.6	35.8	32.8	40.0	35.8
Fujita-Iwataki filtrate					
Somogyi determination	298	214	219	247	170
N.P.N.	30.4	38.7	44.6	35.5	36.2

Table 3. *Comparison of glucose (mg./100 ml. of whole blood) determinations on type A and type C filtrates*

Blood sample ...	1	2	3	4	5	6
Type A filtrate	{ 170 170	80 77	137 138	147 147	130 130	77 95
Type C filtrate	{ 175 175	75 77	137 —	— 146	126 137	93 —

Table 4. *Blood-glucose levels in fowls of various ages*

These are mainly C-type filtrates. A few determinations were done on A filtrates and some on both A and C types.

Group	Sex	No. in group	Age (months)	Fed or fasting	Individual values for glucose (mg./100 ml. of whole blood)	Average
1	F.	16	4-6	Fed	195, 196, 200, 213, 213, 215, 215, 215, 218, 220, 225, 226, 235, 235, 243, 243	219.19
2	F.	15	12-23	Fed	166, 182, 190*, 192*, 195, 198*, 200, 206, 207, 210*, 212, 212, 214*, 217, 225*	201.73
3	F.	20	24-35	Fed	168*, 175, 187*, 192, 192, 192, 193, 194, 196*, 208, 210, 212, 212*, 213, 218*, 218, 222*, 222, 222*, 230	203.80
4	F.	15	36-60	Fed	148, 158*, 164, 170, 170, 178, 185, 187, 188, 195, 197, 205, 215, 218, 234*	187.47
5	M.	9	4-6	Fed	177, 190, 190, 200, 201, 210, 213, 215, 219	201.67
6	M.	7	12-24	Fed	134, 147, 150, 155, 165, 171, 184	158.00
7	M.	5	12-24	Fasted, 48 hr.	77, 95, 130, 138, 147	117.40

* Denotes values for laying hens.

The groups are clearly heterogeneous, only groups 2 and 3 showing no significant difference between them. *t*-Tests show the following differences to be significant at the 1% level: males (groups 5 and 6) have a lower blood glucose than females (groups 1 and 2); young females (group 1 against group 2 and groups 2 and 3 against group 4) have a higher blood glucose than older females: similarly, young males (group 5) have a significantly higher blood glucose than older males (group 6). Fasted males (group 7) have a lower blood glucose than unfasted males (group 6), the *t* value lying between the 1 and 2% levels of probability. There is no difference between the blood-glucose levels of laying (mean 204.15) and non-laying (mean 202.18) hens.

Blood-glucose levels of fowls according to sex and age. The results from seventy birds are in Table 4. The average glucose level tends to be lower in males than in females, and in both sexes a decrease accompanies ageing. In cocks, fasting for 48 hr. lowered the level considerably below that of the fed animal. In the hen, laying did not appear to be accompanied by any characteristic blood-glucose level.

DISCUSSION

As Sturkie (1954, 1955) has pointed out, there are considerable variations in the values reported by different workers for the reducing sugar of fowl's blood (cf. Table 1). In some instances, the value of exhaustive surveys is greatly lessened because one or more factors are omitted from the data. For example age, condition and sex may not be taken into consideration, despite early indications of their importance (e.g. Gioja, 1912).

Since we know very little about the *restreduktion* materials in avian blood and how they affect the reagents employed in reducing-power determinations, it seems neither possible nor profitable to speculate about the wide scatter of the individual and average values of Table 1. The results presented above lead to the following conclusions: (1) Fowl blood has a much higher true glucose level than mammalian blood; no sugar other than D-glucose could be found. (2) In both sexes, the glucose level tends to decrease with age, as previously suggested by Gioja (1912), Heller & Pursell (1937) and Batt

(1939). This may be due to a slow rise in erythrocyte volume with age, as indicated by unpublished observations in this Laboratory. Cocks tend to have a lower blood sugar than hens. This is partly, if not wholly, due to the higher erythrocyte volume in the male (Newell & Shaffner, 1950). Unpublished results from this Laboratory have shown that fowl erythrocytes contain little or no glucose (cf. Tapper & Kare, 1956). (3) Ovulation, or laying, cannot be correlated with the glucose level of the hen's blood, in agreement with Heller & Pursell (1937). (4) Although previous workers have used methods that do not solely determine reducing sugars, their findings are none the less of the same general order as those with the benzidine method. This may be due to 'compensation' by non-sugar blood constituents, some of which may cause 'over-reduction' and others 'under-reduction'. In our experience, blood filtrates made by several methods contain ionizable substances which can cause over- or under-reduction of Somogyi's (1952) reagent.

SUMMARY

1. Use of the stabilized benzidine reagent of Jones & Pridham (1954) on suitable filtrates of fowl blood enables the D-glucose content to be determined colorimetrically, as checked by D-glucose oxidase. The method is simple and the colour produced is stable for at least 24 hr.

2. The average blood-glucose levels of groups of fed hens falls from approximately 220 mg./100 ml.

at 4-6 months to approximately 190 mg./100 ml. at 4-6 years.

3. The average blood glucose of cocks is lower than that of hens, probably owing to greater erythrocyte volumes in the former. It falls from approximately 200 mg./100 ml. at 4-6 months to approximately 160 mg./100 ml. at 1-2 years, and is considerably lowered by fasting.

4. Blood 'filtrates' prepared according to both Somogyi (1945) and Fujita & Iwataki (1931) gave high results with Somogyi's (1952) reagent.

5. No sugars other than D-glucose could be detected in fowl blood.

6. Previous work in this field is summarized and briefly criticized.

REFERENCES

- Bacon, J. S. D. & Bell, D. J. (1948). *Biochem. J.* **42**, 397.
 Batt, H. T. (1939). *Amer. J. Physiol.* **126**, 429.
 Baudoin, A. & Lewin, J. (1927). *Bull. Soc. Chim. biol., Paris*, **9**, 280.
 Bell, D. J. (1947). *J. chem. Soc.* p. 1461.
 Bell, D. J. & Manners, D. J. (1952). *J. chem. Soc.* p. 3461.
 Benedict, S. R. (1925). *J. biol. Chem.* **44**, 207.
 Best, C., Hoet, J. P. & Marks, H. P. (1926). *Proc. Roy. Soc. B*, **100**, 32.
 Bronkhorst, J. J. & Hall, G. O. (1935). *Poultry Sci.* **14**, 42.
 Burr, G. O. & Tanimoto, T. (1950). *Fed. Proc.* **9**, 157.
 Burrows, W. H., Fritz, J. C. & Titus, W. H. (1935). *J. biol. Chem.* **110**, 39.
 Cassidy, G. J., Dworkin, S. & Finney, W. H. (1926). *Amer. J. Physiol.* **75**, 609.
 Cavett, J. W. (1939). *Poultry Sci.* **18**, 150.
 Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
 Corkill, B. (1930). *Biochem. J.* **24**, 779.
 Dyer, H. M. & Roe, J. H. (1934). *J. Nutr.* **7**, 623.
 Erlenbach, F. (1938). *Z. vergl. Physiol.* **26**, 121.
 Folin, O. (1929). *J. biol. Chem.* **82**, 83.
 Folin, O. & Malmros, H. (1929). *J. biol. Chem.* **83**, 115.
 Folin, O. & Wu, H. (1919). *J. biol. Chem.* **38**, 81.
 Fujita, A. & Iwataki, D. (1931). *Biochem. Z.* **242**, 43.
 Gioja, J. (1912). *C.R. Soc. Biol., Paris*, **73**, 102.
 Golden, W. R. C. & Long, C. N. H. (1942). *Endocrinology*, **30**, 675.
 Gonzago, A. (1935). *Rep. N.Y. St. vet. Coll.* 1933-34, p. 53.
 Gulland, J. M. & Peters, R. A. (1930). *Biochem. J.* **24**, 91.
 Hagedorn, J. C. & Jensen, B. N. (1923). *Biochem. Z.* **135**, 46.
 Hayden, C. E. (1929). *Rep. N.Y. St. vet. Coll.* 1927-28, p. 213.
 Hayden, C. E. & Sampson, J. E. (1931). *Rep. N.Y. St. vet. Coll.* 1929-30, p. 174.
 Heller, V. G. & Pursell, L. (1937). *J. biol. Chem.* **118**, 549.
 Henry, K. M., Magee, H. E. & Reid, E. (1934). *J. exp. Biol.* **11**, 58.
 Herbert, F. K. & Bourne, M. C. (1930). *Biochem. J.* **24**, 299.
 Hogan, A. G., Shrewsbury, C. L. & Kempton, H. L. (1928). *Res. Bull. Mo. agric. exp. Sta.* no. 124.
 Horvath, A. A. (1930a). *Poultry Sci.* **9**, 313.
 Horvath, A. A. (1930b). *Amer. J. Physiol.* **94**, 65.
 Hsu, P. T. & Combs, G. F. (1952a). *Arch. Biochem. Biophys.* **38**, 29.
 Hsu, P. T. & Combs, G. F. (1952b). *J. Nutr.* **47**, 73.
 Huggett, A. St G. (1956). *J. Physiol.* **132**, 3 P.
 Hunter, G. (1956). *Biochem. J.* **62**, 28 P.
 Jones, J. K. N. & Pridham, J. B. (1954). *Biochem. J.* **58**, 288.
 Keilin, D. & Hartree, E. F. (1948). *Biochem. J.* **42**, 230.
 Kingsley, G. R. & Reinhold, J. G. (1949). *J. Lab. clin. Med.* **34**, 713.
 Krasnianski, L. & Dsikowski, W. (1931). *Biochem. Z.* **237**, 282.
 Macowan, M. & Magee, H. E. (1932). *Quart. J. exp. Physiol.* **21**, 276.
 Merten, R. (1938). *Biochem. Z.* **297**, 304.
 Meyer, P. (1913). *Biochem. Z.* **50**, 362.
 Murray, H. C. & Rosenberg, M. M. (1953). *Poultry Sci.* **32**, 805.
 Newell, G. W. & Shaffner, C. S. (1950). *Poultry Sci.* **29**, 78.
 O'Neill, A. N. (1955). *Canad. J. Chem.* **33**, 1097.
 O'Neill, A. N. & Hawkins, W. W. (1955). *Canad. J. Biochem. Physiol.* **33**, 545.
 Opdyke, D. F. (1942). *Endocrinology*, **31**, 363.
 Rogement, L. (1930). *C.R. Soc. Biol., Paris*, **104**, 154, 256.
 Rothberg, V. E. & Evans, F. A. (1923). *J. biol. Chem.* **58**, 431.
 Russell, W. C. & Weber, A. L. (1934). *Poultry Sci.* **13**, 376.
 Scheunert, A. & Pelchriz, H. von (1923). *Biochem. Z.* **139**, 17.
 Schwartz, K. & Heinrich, K. (1928). *Biochem. Z.* **194**, 346.
 Shaffer, P. A. & Hartman, A. F. (1921). *J. biol. Chem.* **45**, 344.
 Somogyi, M. (1930). *J. biol. Chem.* **86**, 655.
 Somogyi, M. (1945). *J. biol. Chem.* **160**, 69.
 Somogyi, M. (1952). *J. biol. Chem.* **195**, 19.
 Strange, R. E., Dark, F. A. & Ness, A. G. (1955). *Biochem. J.* **59**, 172.
 Sturkie, P. D. (1954). *Avian physiology*. Ithaca: Comstock Publ. Associates.
 Sturkie, P. D. (1955). *Endocrinology*, **56**, 575.
 Tapper, D. N. & Kare, M. R. (1956). *Proc. Soc. exp. Biol., N.Y.*, **92**, 120.
 Thompson, T. J. & Carr, I. L. (1923). *Biochem. J.* **17**, 373.