The **230th Meeting** *of the Biochemical Society was held at St Thomas's Hospital Medical School, Manor House, Godalming, Surrey, on Saturday, 25 September* 1943, *at* 12 *noon, when the following papers were read:*

Further Observations on the *C. diphtheriae* Growth Factor of the B Complex. By F. W. CHATTAWAY, F. C. HAPPOLD and MARY SANDFORD

The paper constitutes a further report on the properties of the *C. diphtheriae* growth factor present in the amyl-alcohol insoluble residues of extracts of whole liver.

The factor is relatively stable to acid, and is markedly labile to alkali, nitrous acid, ninhydrin and hydrogen peroxide. Methylation and acetylation are highly destructive and activity cannot be regained by acid hydrolysis. Adsorption occurs on norite charcoal but not on Fuller's earth. Extraction cannot be effected by diethyl ether, chloroform, petroleum ether, amyl alcohol, butyl alcohol or *p*-cresol at determined *p*H values. Precipitation of the active component does not occur with silver salts at acid and neutral reactions, and occurs only slightly with silver sulphate in the presence of baryta at *p*H values above 8·0.

Lead acetate is ineffective as a precipitating agent at *p*H values of 2·8, 6·9 and 9 to 10.

Phosphotungstic acid in 5 % sulphuric acid removes inert material from solution.

The activity of the most purified preparations is such that the addition of 5γ dried material per ml. of basal medium promotes moderate growth whereas one-hundredth of this amount evokes a detectable growth response. It is free from all traces of *L. casei* ϵ growth factor.

A Tissue Growth-Promoting Factor present in Pancreatin. By J. N. DAVIDSON and C. WAYMOUTH

Commercial pancreatin contains a factor which stimulates the growth in roller tubes by Willmer's* technique of fresh explants of the 9-day chick embryo heart.† When the factor is added along with embryo extract to roller tubes containing six fresh explants (embedded in fowl plasma) which have been cultivated in Tyrode solution for 48 hr., it produces in the course of a further 48 hr. a much greater rise in nucleoprotein phosphorus (N.P.P.) in the cultures than does embryo extract alone.

Composition of fluid phase in roller tubes in second 48 hr. of test	Change in N.P.P. μg. per roller tube
Embryo extract alone	+0·36
Embryo extract + crude factor	+0·82
Embryo extract + factor heated at 100° for 30 min. at *p*H 10	+0·39
Tyrode solution alone	−0·09
Purified factor (N = 50 mg./100 ml.) in Tyrode *without* embryo extract	+0·66

The factor is stable to heat in neutral or slightly acid solution, but is inactivated by heating in faintly alkaline solution. It can be purified by extraction with phenol and the purified material exerts a powerful growth-promoting action on chick heart fibroblasts even in the absence of embryo extract.

* Willmer, E. N. [1942]. *J. exp. Biol.* **19**, 11.

† Davidson, J. N. & Waymouth, C. [1943]. *Biochem. J.* **37**, 271.

Studies on the Plasma Fibrinogen and other Protein Fractions in a Case of Afibrinogenaemia. By J. L. PINNIGER and F. T. G. PRUNTY

By adaptation of the Kark and Soutar* prothrombin technique, and from precipitin studies, evidence is presented for complete lack of fibrinogen in a girl with haemorrhagic

symptoms. The relation of the *in vitro*† and *in vivo* fibrinogen to the formation of clots, and the effects of transfusion of concentrated plasma on the protein fractions of the patient's plasma have been investigated.

* Kark & Soutar [1940]. *Lancet*, **1**, 1149.
† Witts, L. J. [1942]. *J. Path. Bact.* **54**, 516.

Fluorescent Derivatives of N-Methyl-Chloro-Nicotinamide. By R. A. COULSON and P. ELLINGER

The supposition that F_2 might be thiochrome* proved to be incorrect.† The analogy with the elimination of methylated products after the intake of pyridine‡ and nicotinic acid§ suggested the possibility of the formation of methyl derivatives of nicotinamide which might be related to F_2.†‖ The natural non-fluorescent precursor of F_2 was crystallized from KCl eluates of urine rich in the compound by low temperature vacuum distillation, fractional precipitation of KCl and other impurities, and by adsorption on Al_2O_3 and elution with methanol. An orange aurate (m.p. 164°), a yellow mercury-potassium iodide (m.p. 136·3°) and a yellow picrate (m.p. 190·2°) were formed from the concentrate. The melting-points of the latter two were identical to the corresponding salts of synthetic N-methyl-chloro-nicotinamide†¶ and the mixed melting-points showed no depression. The aurate differed in melting-point and colour (synthetic: 170·2° yellow) (possibly two aurates as in trigonelline).**

The transformation by alkali of N-methyl-chloro-nicotinamide into two fluorescent compounds F_{2a} and F_{2b}† is nearly complete in dry isobutanol, whereas in water it is incomplete and the destruction is rapid (cf. formation of a carbinol from N-methyl-pyridium hydroxide††). The maximum yield by extraction of the alkaline aqueous phase by isobutanol is only 20 %. The predominance of the quaternary ammonium compound in water and of the carbinol† in isobutanol is evident from the spectrographs (prepared by Dr E. M. F. Roe), the shift of which resembles that obtained for the non-reduced and reduced N-methyl-iodo-nicotinamide‡‡ respectively.

* Coulson, R.A., Ellinger, P. & Platt, B. S. [1942]. *Biochem. J.* **36**, xii.
† Ellinger, P. & Coulson, R.A. [1943]. *Nature, Lond.* (in the Press).
‡ His, W. [1887]. *Arch. exp. Path. Pharmak.* **22**, 253. Cohn, R. [1893]. *Hoppe-Seyl. Z.* **18**, 16; and many others.
§ Ackermann, D. [1913]. *Z. Biol.* **59**, 18. Linneweh, W. & Reinwein, H. [1932]. *Hoppe-Zeyl. Z.* **207**, 48; **209**, 110.
‖ Najjar, V. A., Scott, D. B. M. & Holt, jr., L.E. [1943]. *Science*, **97**, 537. Huff, J. W. & Perlzweig, W. A. [1943]. *Science*, **97**, 538.
¶ Karrer, P., Schwarzenbach, G., Benz, F. & Sollmsen, V. [1936]. *Helv. Chim. Acta*, **19**, 826.
** Jahns, E. [1885]. *Ber. dtsch. chem. Ges.* **18**, 2518; [1887] **20**, 2840.
†† Hantzsch, A. & Kalb [1899]. *Ber. dtsch. chem. Ges.* **32**, 3117; Decker, E. & Kaufmann, A. [1911]. *J. prakt. Chem.* [2] **84**, 432.
‡‡ Warburg, O. & Christian, W. [1936]. *Biochem. Z.* **287**, 315.

The Excretion of Histidine in Urine. By J. R. O'BRIEN and P. E. QUELCH

Studies in the metabolism of histidine* in pregnancy have raised problems, the solution of which would be aided by a sound method of estimation. One group of methods uses Knoop's reaction for this amino-acid which, although almost specific for histidine,† is sensitive to urinary constituents such as uric acid, phosphate and urea. To eliminate interfering substances a method based upon a base-exchange between histidine and permutite was worked out for urine. Acidified urine is allowed to percolate through a column of permutite, which is then washed with water and eluted with a saturated solution of KCl. The concentration of the purple product of Knoop's reaction on the eluate is measured photometrically.

Results obtained by this method were: Normal individuals differed greatly in the daily amounts of urinary histidine excreted—values from 0–400 mg. per day were obtained. The excretion of histidine fluctuated throughout the day and seemed related to the urinary output. Early morning specimens of urines from normals contained 0–30 mg./100 ml. In pregnancy, early morning specimens contained usually 30–100 mg./100 ml. In pregnancy complicated by toxaemia, threatened abortion or diabetes, the urinary histidine fell to values below the normal. From the present data, it would seem that estimation of urinary histidine is a useful rather than a reliable diagnostic aid in pregnancy.

* Kapeller-Adler, R. & Adler, E. [1943]. *J. Obstet. Gynaec.* **50**, 177.
† Armstrong, A. R. & Walker, E. [1932]. *Biochem. J.* **26**, 143.

The Osmotic Pressure of Foetal Haemoglobin. By E. F. McCarthy and G. Popják

The oxygen dissociation curves of foetal and maternal haemoglobin solutions with identical concentrations of hydrogen ions and salts, have been shown to differ markedly in position. In addition there seems to be a slight difference in the shape of the curves which may be represented by the calculation of n in Hill's equation $y/100 = kx^n/(1+kx^n)$, in which y represents percentage saturation, x oxygen tension and k is a constant. The value of n for foetal haemoglobin is usually slightly lower than that for maternal haemoglobin and it seemed desirable therefore to compare the degree of aggregation of foetal and maternal haemoglobin by osmotic pressure measurements.

Oxygen dissociation curves were measured on solutions of foetal and maternal haemoglobin prepared from the corresponding bloods of sheep at an early stage of pregnancy. Low values of n were obtained for foetal haemoglobin. Osmotic pressure measurements made by Adair's method showed no significant difference between foetal and maternal haemoglobin.

The Osmotic Pressure of 'Defatted' Serum. By G. Popják and E. F. McCarthy

Fresh, pooled human serum was 'defatted' by the method of McFarlane, i.e. by shaking it with ether, then freezing the mixture to below $-25°$ and thawing. The osmotic pressure of serum extracted five times in this way was compared with that of the untreated serum. The osmotic pressure measurements were made by the method of Adair. The results showed that the defatted serum exerts slightly higher osmotic pressure per gram of protein than the whole serum. However, this difference can be explained by the increase in the albumin-globulin ratio of the serum after the extraction. The conclusions are drawn that (*a*) extraction of serum by McFarlane's method removes a portion of the globulin complex, but does not alter the colloid osmotic pressure properties of the remaining proteins; and (*b*) the lipids extracted from the serum have no effect on the osmotic pressure of serum proteins.

Alloxan Diabetes (Shaw Dunn) in the Rabbit. By L. L. Ware (introduced by F. G. Young)

Vitamin C Nutrition. The Correlation of Plasma Ascorbic Acid Concentration and Urinary Saturation Tests. By F. T. G. Prunty and C. C. N. Vass

Unless the subject has recently been on an appreciably higher intake of ascorbic acid than he is now receiving, the plasma ascorbic acid concentration is a satisfactory index of the nutritional state with respect to vitamin C. Moreover, this determination is more easily carried out than a saturation test. When a 'state of saturation' is attained the plasma ascorbic acid concentration is greater than 0·8 mg./100 ml. For clinical purposes it is

suggested that a concentration of not less than 0·4 mg./100 ml. should be considered as a normal value. In general the results obtained by this determination are in agreement with those of Harris* in recent surveys.

* Harris, L. J. [1943]. *Lancet*, **1**, 515.

Succinyl Sulphathiazole and Coprosterol Formation. By O. ROSENHEIM and T. A. WEBSTER.

The bacteriostatic action of succinyl sulphathiazole on intestinal coliform bacteria was found to be concurrent with a complete inhibition of coprosterol formation. The drug was administered to rats in a diet on which about 80 % of the total sterols are excreted as coprosterol.* The conclusion that coliform bacteria are responsible for coprosterol formation was negatived by the fact that, in spite of the immediate reappearance of *B. coli* on withdrawal of the drug, the faecal sterols were found to contain only traces of coprosterol.

These and other results throw doubt on the assumed role of intestinal bacteria and suggest the possibility that the fauna rather than the flora of the intestine may be concerned in coprosterol formation. Protozoa and especially species of *trichomonas* are common parasites of the intestine of man and animals and cholesterol has been recognized as an essential growth factor for four species of *trichomonas*.†

We found that carbarsone (*p*-carbamino phenylarsonic acid), which frees the intestine from *trichomonas*, also completely inhibits coprosterol excretion. The correlation, however, appeared to be fortuitous, for rats freed from *trichomonas* by carbarsone excreted considerable amounts of coprosterol on withdrawal of the drug, but only small amounts some time after treatment with succinyl sulphathiazole, which had not freed them of the flagellates. This interference with colesterol metabolism, shared by an antibacterial and an amoebicidal drug, seems to be unconnected with their action on either bacteria or protozoa and remains so far unexplained.

* Rosenheim, O. & Webster, T. A. [1941]. *Biochem. J.* **35**, 920.

† Cailleau, R. (1937). *Ann. Inst. Pasteur*, **59**, 137, 293; (1939) *C.R. Soc. Biol., Paris*, **130**, 1089.

A Study of the Accuracy of Haemoglobin Methods. By E. J. KING, MARGARET GILCHRIST and G. E. DELORY

Three colorimetric haemoglobin methods—alkaline haematin, cyanmethaemoglobin and carboxyhaemoglobin—were tested by comparison with Fe analyses and O_2 capacity determinations. A Leitz colorimeter (Duboscq type) with mercury green light and a photoelectric colorimeter with a Chance green filter were used, with Ilford grey screens as standards. For the alkaline haematin method a haemin standard was also used. Good results were obtained on each colorimeter; photoelectric readings entail less labour. The variation of each specimen from the average colorimetric relation to iron or oxygen was calculated, and these results expressed as standard deviations.

Twelve haemoglobin preparations from horse and human bloods were found to give colours in NaOH agreeing with their iron content, when compared with standard haemin solutions.

On 20 normal bloods the standard deviations from Fe analyses and O_2 capacities were: alkaline haematin, 2·33 and 2·26 %; cyanmethaemoglobin, 1·25 and 2·18 %; carboxyhaemoglobin, 1·70 and 2·49 %; Haldane dilution tube, 3·02 %. On 20 specimens of washed laked cells from miscellaneous hospital bloods the standard deviations were: alkaline haematin, 3·30 and 2·56 %; cyanmethaemoglobin, 2·63 and 2·11 %; carboxyhaemoglobin, 4·14 and 3·32 %.

The cyanmethaemoglobin method is the most accurate; the other two give accuracy satisfactory for most purposes. The alkaline haematin method is preferred for routine work.