maximum decrease in the proportion of water to dry matter in the particles being 29% in 15 min. with DPNH (6·7 mM) and 18·5% with TPNH (12·8 mM). During shrinkage, there was a selective decrease in the 'chloride space' of the mitochondria.

4. Loss of added nucleotides was always slight at 0°, the greatest amounting to 18% disappearance of DPNH (originally 2·4 mM) in 30 min. The maximum rate of loss was approximately trebled when the temperature was raised to 25°.

5. TPN^+ and TPNH were dephosphorylated, the former more rapidly; the products appeared in both the mitochondria and supernatant fluid.

6. DPNH was oxidized slowly (approximately 0·032 μmole/mg. dry wt./hr.) in the suspension, but much more rapidly when the mitochondria were sedimented. The rate of oxidation of TPNH under similar conditions was approximately two-thirds of the corresponding values for DPNH.

7. After addition of DPN^+, DPNH, TPN^+ and TPNH at concentrations greater than 5 mM to different suspensions, the mitochondria contained DPN^+ and DPNH at concentrations 0–25% and TPN^+ and TPNH at concentrations 37–41% of that in the supernatant fluid. This difference in 'permeability' was demonstrated over a wide range (2–15 mM) of concentrations and at both 0° and 25°. Only DPNH was adsorbed by the mitochondria.

8. TPN^+ and TPNH permeating the mitochondria enter the 'pool' of functional nucleotides.

9. Degenerative changes in the original mitochondrial preparations (swelling, low level of endogenous reduced nucleotides and inability to maintain reduced nucleotides) resulted in a greatly increased 'permeability' to the diphosphopyridine nucleotides.

We wish to thank Professor Sir Hans Krebs, F.R.S., for his interest in the work, and Miss B. M. Notton and Mr R. Ker for technical assistance. The work, during which an 1851 Scholarship was held by one of us (L. M. B.), was aided by a grant from the Rockefeller Foundation.

REFERENCES

Allard, C., de Lamirande, G., Faria, H. & Cantero, A. (1954). *Canad. J. Biochem. Physiol.* **32**, 383.
Amoore, J. E. (1958). *Biochem. J.* **70**, 718.
Amoore, J. E. & Bartley, W. (1958). *Biochem. J.* **69**, 223.
Baessler, K. H. & Pressman, B. C. (1959). *Fed. Proc.* **18**, 184.
Bassham, J. A., Birt, L. M., Hems, R. & Loening, U. L. (1959). *Biochem. J.* **73**, 491.
Chance, B. & Baltscheffsky, H. (1958). *J. biol. Chem.* **233**, 736.
Chance, B. & Williams, G. R. (1955). *J. biol. Chem.* **217**, 395.
Chappell, J. B. & Perry, S. V. (1954). *Nature, Lond.*, **173**, 1094.
Dickens, F., Glock, G. E. & McLean, P. (1959). *Ciba Foundation Symp., Regulation of Cell Metabolism*, p. 183. Ed. by Wolstenholme, G. E. W. & O'Connor, C. M. London: J. and A. Churchill Ltd.
Glock, G. E. & McLean, P. (1956). *Exp. Cell Res.* **11**, 234.
Hunter, F. E., Malison, R., Bridgers, W. F., Schutz, B. & Atchison, A. (1959). *J. biol. Chem.* **234**, 693.
Jacobson, K. B. & Kaplan, N. O. (1957*a*). *J. biol. Chem.* **226**, 603.
Jacobson, K. B. & Kaplan, N. O. (1957*b*). *J. biophys. biochem. Cytol.* **3**, 31.
Joshi, S., Newburgh, R. W. & Cheldelin, V. H. (1957). *J. biol. Chem.* **229**, 771.
Lehninger, A. L. (1951). *J. biol. Chem.* **190**, 345.
Lehninger, A. L. (1955–56). *Harvey Lect.* **49**, 176.
Lehninger, A. L., Ray, B. L. & Schneider, M. (1959). *J. biophys. biochem. Cytol.* **5**, 97.
Lehninger, A. L., Wadkins, C. L., Cooper, C., Devlin, T. M. & Gamble, J. L. (1958). *Science*, **128**, 450.
Maley, G. F. (1957). *J. biol. Chem.* **224**, 1029.
Maley, G. F. & Lardy, H. A. (1955). *J. biol. Chem.* **215**, 377.
Purvis, J. L. (1958). *Nature, Lond.*, **182**, 711.
Racker, E. (1950). *J. biol. Chem.* **184**, 313.
Sanderson, P. H. (1952). *Biochem. J.* **52**, 502.
Singer, T. P. & Kearney, E. B. (1950). *J. biol. Chem.* **183**, 409.
Sung, S. & Williams, J. N. (1952). *J. biol. Chem.* **197**, 175.
Werkheiser, W. C. & Bartley, W. (1957). *Biochem. J.* **66**, 79.

Biochem. J. (1960) **75**, 315

Lipid Synthesis by Human Leucocytes *in vitro*

By A. A. BUCHANAN*
Medical Unit, University College Hospital Medical School, London, W.C. 1

(*Received* 9 *September* 1959)

Synthesis of lipid by human and rabbit erythrocytes *in vitro* has been reported by several investigators (Altman, Watman & Salomon, 1951; Altman 1953; James, Lovelock & Webb, 1957; Marks, Johnson, Hirschberg & Banks, 1958; Rowe, 1959; Turner, 1958). In all these studies blood, sometimes with the 'buffy coat' removed after centrifuging, has been incubated with [^{14}C]acetate. After incubation the cells, plasma or whole blood have been extracted for lipids and this lipid extract

* Present address: Medical Unit, St Mary's Hospital, Paddington, London.

or its fractions has been measured for radioactivity. The erythrocyte lipids are labile and readily removed by washing (Lovelock, 1955), and they appear to decrease as the cell ages (Prankerd, 1958).

The erythrocyte lipid is mainly structural in function (Ponder, 1954) and it is thought that the function of the lipid synthesis is to maintain the integrity of the erythrocyte membrane. Marks *et al.* (1958) have shown that, if erythrocytes are centrifuged, the top young cells have a greater ability to synthesize lipid than the bottom older cells.

During an attempt to measure quantitatively the rate of lipid synthesis by blood cells, it became apparent that duplicate readings on the same blood samples gave very different rates of incorporation of radioactivity from [^{14}C]acetate into the lipid fraction. In agreement with Marks *et al.* (1958) it was observed that, after centrifuging, the top layers of cells gave considerably higher readings than the bottom layers of cells, the bottom cells on some occasions showing complete inactivity.

Since removal of the 'buffy' layer after centrifuging removes only 60–80% of the leucocytes (McKane & Ingram, 1957), leaving the remaining leucocytes mainly in the upper layers of the erythrocytes (Burt & Rossiter, 1950), and since lipid synthesis by mature erythrocytes seems unlikely in view of their lack of respiratory enzymes (Granick, 1949) or lipase (Valentine, 1958), it was thought that a possible explanation of the lack of reproducibility of results could be that the lipid synthesis is performed mainly or entirely by the white cells.

METHODS

General conditions. All blood samples were taken from normal healthy volunteers. Glassware and syringes were treated with silicone and heparin or ethylenediaminetetra-acetic acid or both were used as anticoagulants. Whether heparin or ethylenediaminetetra-acetic acid was used made no difference to the experimental results. Blood samples were kept chilled during all manipulations until incubated. All cells were incubated in a measured sample of their own plasma (previously obtained by centrifuging) at 37°, being gently agitated throughout.

Preparation of leucocytes. Leucocytes were prepared by dextran sedimentation (Davis, Spun & Wilson, 1958), centrifuging (Jago, 1956; McKane & Ingram, 1957) or by haemolysis of erythrocytes with saponin (Schwartz, Grazia, Cheney, Hasenfus & Cohen, 1956). By the last-named method the total leucocytes from 1 ml. samples of blood were obtained, the erythrocyte haemolysis being performed with the minimum concentration of saponin necessary in cold aqueous 0·6% NaCl. Control total cell samples from similar 1 ml. samples of the same blood were washed with the same concentration of saponin in cold aqueous 0·9% NaCl (this does not haemolyse the erythrocytes).

Preparation of erythrocytes. Erythrocytes have not been obtained completely free from leucocytes, but the leucocyte count can be reduced to less than 50/mm.^{3} (total packed cell volume 50%) by filtration of the blood through cotton wool (Fleming, 1926) and repeatedly centrifuging and discarding the upper layers of cells (Munn, 1958).

By these methods reticulocytes and very young cells are discarded, but it has been found impossible to separate reticulocytes from leucocytes.

Labelling and measurement of radioactivity. Sodium [^{14}C]acetate (68 μc/mg., approx. 1 μc/ml., or [^{32}P]orthophosphate (>1 c/mg.), 100 μc/ml., was added to the cells in plasma just before incubation, the exact number of counts added being determined by counting a measured sample of the plasma immediately after mixing. All counting was at infinite thickness with a thin end-window counter (1·7 mg./cm.^{2}), counting being done for a sufficient time to give an accuracy of ± 4% in all except the least-active samples.

Separation of lipids. After incubation cells were centrifuged and washed once with aqueous 0·9% NaCl. The total lipid was extracted from the cells and the plasma by the method of Folch, Lees & Sloane-Stanley (1957); the extract was evaporated to small volume under reduced pressure and transferred to planchettes. The lipid was weighed on the planchettes.

RESULTS

All radioactivity has been expressed as counts/mg. of lipid extracted/min. and corrected to 10 000 counts/ml./min. added to the plasma before incubation.

The amounts of lipid extracted from plasma and erythrocytes agreed very well with reports of previous investigators [erythrocytes approx. 5 mg./ml. of cells (Wintrobe, 1956); plasma approx. 8 mg./ml. (Peters & van Slyke, 1946)]. The leucocyte lipid was found to be approx. 19 mg./ml., slightly higher than that reported by Boyd (1936) but agreeing with the value given by Burt & Rossiter (1950).

It was found that in all cases leucocytes were 100–1000 times more active in the uptake of radioactivity into their lipid fraction from the labelled acetate than were erythrocytes. In a few experiments where erythrocytes have been obtained uncontaminated by leucocytes (less than 50 mm.^{3}), the erythrocytes have been found to be completely inactive.

Figs. 1 and 2 show the results of typical experiments. The labelled lipid was found both in the cells and the plasma in which the cells were incubated. The rate of labelling of the lipid was initially extremely rapid, the labelling being detectable after 2 min. in both the cells and plasma (Fig. 2). This rate of labelling began to fall after incubation for about 3–4 hr., and after about 6 hr. very little further increase in uptake of ^{14}C into the lipid fraction occurred (Fig. 1). Incorporation of [^{14}C]-acetate into the lipid fraction of the leucocytes is shown approx. 150 times that of the erythrocytes

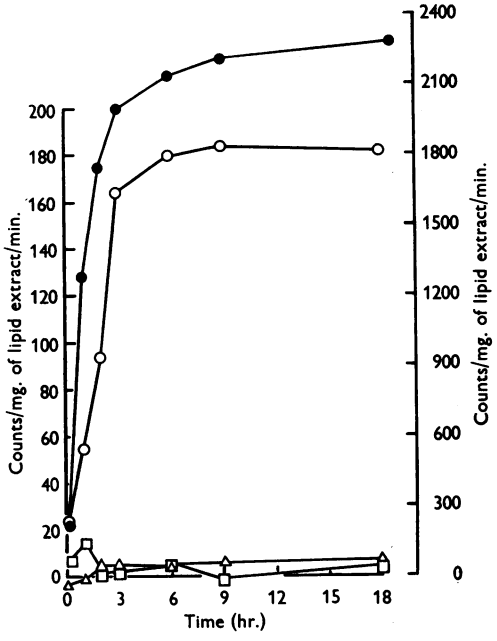

Fig. 1. Incorporation of [1-^{14}C]acetate into the lipid extract from leucocytes (●) and the plasma in which the leucocytes were incubated (○), and from erythrocytes (△) and the plasma in which the erythrocytes were incubated (□). Counts obtained from lipid extracts are plotted against time of incubation at 37° after addition of sodium [1-^{14}C]acetate. Leucocyte counts are on a scale (right-hand ordinate) 10 times that for the counts of leucocyte plasma, erythrocyte and erythrocyte plasma (left-hand ordinate). All counts are corrected to 10 000 counts/ml./min. added to the plasma before incubation.

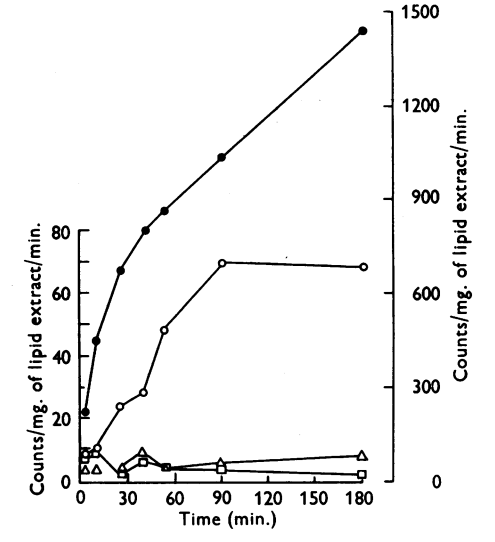

Fig. 2. Details are as given for Fig. 1. The first points are measured 2 min. after addition of the [1-^{14}C]acetate.

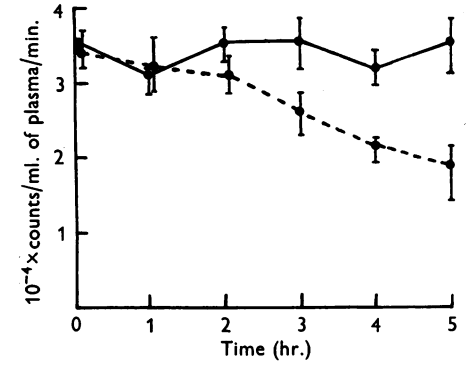

Fig. 3. Loss of [1-^{14}C]acetate from the plasma in which cells were incubated. Counts obtained from lipid extracts are plotted against time of incubation at 37° after addition of sodium [1-^{14}C]acetate. ●---●, Normal blood (containing leucocytes and erythrocytes; haematocrit value 42%). ●——●, Pure erythrocytes added to plasma to give a haematocrit value of 42%. Equal concentrations of [1-^{14}C]-acetate were added to each sample.

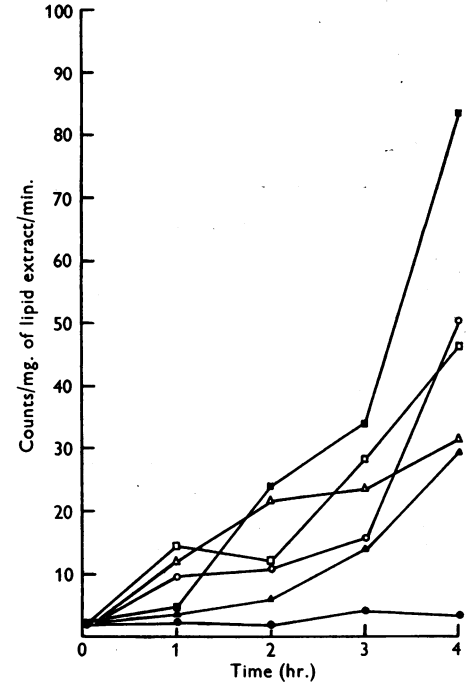

Fig. 4. Incorporation of [1-^{14}C]acetate into the total lipid extract from 1 ml. samples of: ■, normal blood incubated at 37°; ●, normal blood incubated at 4°; ○, normal blood + sodium azide to a final concentration 0·05 M; □, normal blood + dinitrophenol to a final concentration 0·1 mM; △, normal blood + potassium cyanide to a final concentration 0·1 mM; ▲, normal blood + sodium fluoride to a final concentration 0·01 M. Counts obtained from lipid extracts are plotted against time of incubation at 37° after addition of equal quantities of sodium [1-^{14}C]acetate to each sample. All counts are corrected to 10 000 counts/ml./min. added.

since counts have been expressed per mg. of lipid extracted. If the rate of incorporation of [^{14}C]-acetate is corrected for the amount of lipid extracted from erythrocytes and leucocytes (approx. 1:4), the differential rates of incorporation of [^{14}C]acetate become approx. 600:1. Similarly, the plasma counts can be corrected for the differential packed-cell volume between the erythrocytes and leucocytes when the ratio leucocyte-plasma-lipid radioactivity:erythrocyte-plasma-lipid radioactivity in Fig. 1 becomes approx. 50:1.

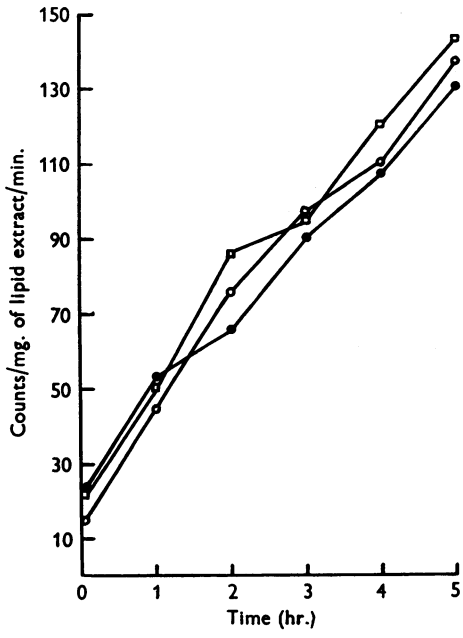

Fig. 5. Incorporation of radioactivity into the total lipid extract from 1 ml. samples of whole blood. ○, [1-^{14}C]-Acetate added to the plasma; □, [2-^{14}C]acetate added to the plasma; ●, [1-^{14}C]acetate + [2-^{14}C]acetate added to the plasma. All counts are corrected to 10 000 counts/min./ml. added to the plasma before incubation. Counts obtained from lipid extracts of whole blood are plotted against time of incubation at 37° after addition of radioactivity.

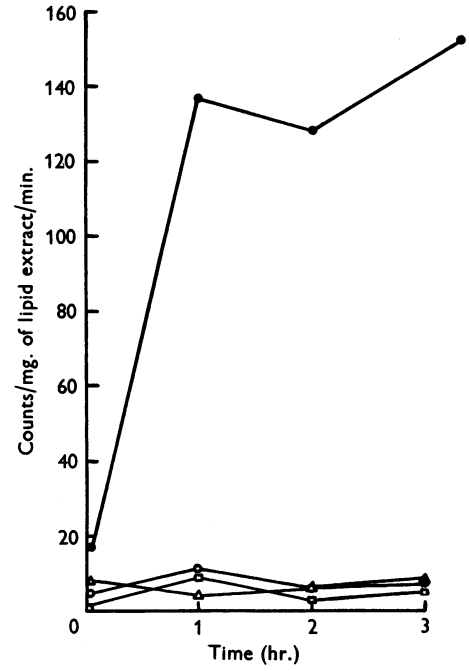

Fig. 6. Incorporation of ^{32}P into the total lipid extract from leucocytes (●) and the plasma in which the leucocytes were incubated (○), and from erythrocytes (△) and the plasma in which the erythrocytes were incubated (□). Counts obtained from lipid extracts are plotted against time of incubation at 37° after addition of [^{32}P]orthophosphate. All counts are corrected to 10 000 counts/min./ml. added to the plasma before incubation.

Table 1. *Synthesis of lipids by samples from whole blood*

Figures represent the weight and radioactivity of lipids extracted from cells and plasma from 1 ml. samples of blood. Erythrocytes have been removed or left in the samples. Samples were incubated for 3 hr. with sodium [1-^{14}C]acetate and lipid extracts prepared before and after incubation.

Sample	Wt. of lipid extract before incubation (mg.) (1 expt.)	Lipid extract before incubation (total counts/min.) (1 expt.)	Wt. of lipid extract after incubation for 3 hr. (mg.) (means of 5 expts.)	Lipid extract after incubation for 3 hr. (total counts/min.) (means of 5 expts.)
Total washed cells from 1 ml. of blood + 0·5 ml. of plasma	5·9	0·8	4·7 (S.D. 1·1)	110·8 (S.D. 20·9)
Total leucocytes from 1 ml. of blood + 0·5 ml. of plasma	3·5	0·8	3·3 (S.D. 0·8)	119·9 (S.D. 12·4)
Plasma (0·5 ml.) from total washed cells from 1 ml. of blood + 0·75 ml. of plasma	3·5	0·3	3·1 (S.D. 0·3)	19·7 (S.D. 1·0)
Plasma (0·5 ml.) from leucocytes of 1 ml. of blood + 0·75 ml. of plasma	3·6	−1·2	3·4 (S.D. 0·5)	21·2 (S.D. 3·2)

Cell-free plasma incubated with [^{14}C]acetate showed no labelling of the lipid fraction.

The total plasma ^{14}C radioactivity fell during the incubation as acetate was utilized by the cells (Fig. 3). Samples containing erythrocytes alone utilized very little, if any, of the [^{14}C]acetate during incubation for 5 hr. (Fig. 3). Calculation for whole blood showed that 2·8% (S.D. 0·65) of the radioactivity disappearing from the plasma appeared in the cell lipids.

Uptake of ^{14}C into the lipid fraction was completely inhibited by incubation at 4° and partially inhibited by glycolytic- or respiratory-enzyme inhibitors, such as sodium fluoride, sodium azide, dinitrophenol and potassium cyanide (Fig. 4).

In order to determine whether erythrocytes can synthesize lipid from acetate the incorporation of [^{14}C]acetate into the lipid fraction of the total leucocytes from 1 ml. samples of blood, obtained by haemolysing the erythrocytes with saponin (Schwartz *et al.* 1956), was compared with the rate of incorporation of the total cells (erythrocytes and leucocytes) from 1 ml. samples of the same blood.

Table 1 shows that after incubation for 3 hr. the lipid extract from both the total samples (cells + plasma) and the plasma alone contained the same radioactivity whether or not the erythrocytes were present.

If sodium [2-^{14}C]acetate was added to blood before incubation in the same quantities as was normally used for [1-^{14}C]acetate the same amount of labelling occurred in the lipid fraction (Fig. 5). Extracts of lipid from leucocytes incubated with [^{32}P]orthophosphate also showed uptake of radioactivity, presumably into their phospholipids (Fig. 6).

DISCUSSION

The rapid metabolic rate of leucocytes is well known; they have $Q_{O_2} - 32{\cdot}6$ μl. of oxygen/mg. dry wt./hr. as compared with Q_{O_2} of liver, $-11{\cdot}6$ μl. (Wagner & Sparaco, 1958), and all metabolic functions studied have confirmed this (Lawrence, 1955; Valentine, 1956; Beck, 1957, 1958; Winzler, 1958). The results reported here show that leucocytes are also very active in lipid synthesis.

It has been shown that in normal blood all the lipid synthesis is carried out by leucocytes, since the same rate of uptake of ^{14}C occurs whether the erythrocytes are present or not. Also erythrocytes appear not to be able to metabolize acetate, which is in agreement with their known lack of Krebs-cycle enzymes. The synthesis of lipid by reticulocytes (Marks & Gellhorn, 1959) is not demonstrated or disproved by the findings, since all blood used had a normal low reticulocyte count and these cells were discarded in the leucocyte and erythrocyte separation.

The lack of complete inhibition of lipid synthesis by enzyme inhibitors agrees with the findings of McKinney & Martin (1956) and McKinney, Martin, Rundles & Green (1953), who showed that sorbose 1-phosphate, oxamic acid, sodium fluoride, potassium cyanide, dinitrophenol and fluoroacetate did not completely depress leucocyte respiration.

The finding of labelled lipid in the plasma in which the leucocytes were incubated could be due either to lipids being released from living leucocytes into the plasma or to lysis of leucocyctes during incubation after they have synthesized labelled lipid. The former is the more likely explanation, since the time course of the appearance of plasma lipid follows that of the cell lipids. If the plasma-labelled lipid were due to death of leucocytes the course of plasma-labelled lipid would be expected to rise as more leucocytes lyse.

If after incubation for 19 hr. a stained smear of the leucocytes is examined, very little debris is seen; the cells all appear intact although their cytoplasm is somewhat amorphous. Unfortunately it is impossible to count the cells before and after incubation since they clump during incubation and attempts to disperse them by agitation result in lysis of large numbers of cells.

Leucocytes remain viable *in vitro* for up to 4 days (Malec & Zakrzewski, 1957). If living leucocytes can lose lipid to the plasma, this may be an important source of the plasma lipid. James *et al.* (1957) have shown that lipid synthesized by whole blood becomes attached to the plasma proteins, mainly to the α-globulins.

SUMMARY

1. Synthesis of lipids by human leucocytes *in vitro* has been demonstrated; the amount of synthesis is sufficient to account for all the lipid synthesized by whole blood.

2. It is probable that all earlier reports of lipid synthesis by erythrocytes were due to leucocyte contamination.

This work was done during the tenure of an Undergraduate Studentship of London University. I am grateful to Dr T. A. J. Prankerd for his encouragement and help, and to Professor M. L. Rosenheim for providing the facilities for this work.

REFERENCES

Altman, K. I. (1953). *Arch. Biochem. Biophys.* **42**, 478.
Altman, K. I., Watman, R. N. & Salomon, K. (1951). *Arch. Biochem. Biophys.* **33**, 169.
Beck, W. S. (1957). *Univ. Calif., Los Angeles: Sch. Med. Atomic Energy Rep.* no. 414.
Beck, W. S. (1958). *Ann. N.Y. Acad. Sci.* **75** (1), 4.
Boyd, E. M. (1936). *Arch. Path.* **21**, 739.

Burt, N. S. & Rossiter, R. J. (1950). *Biochem. J.* **46**, 569.
Davis, V. E., Spun, C. L. & Wilson, W. L. (1958). *Blood*, **13**, 367.
Fleming, A. (1926). *Brit. J. exp. Path.* **7**, 281.
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Granick, S. (1949). *Blood*, **4**, 404.
Jago, M. (1956). *Brit. J. Haematol.* **2**, 439.
James, A. T., Lovelock, J. E. & Webb, J. (1957). *Biochem. J.* **66**, 60P.
Lawrence, J. S. (1955). *J. Amer. med. Ass.* **157**, 1212.
Lovelock, J. E. (1955). *Biochem. J.* **60**, 692.
McKane, A. & Ingram, M. (1957). *Univ. Rochester Atomic Energy Proj.* no. 490.
McKinney, G. R. & Martin, S. P. (1956). *Blood*, **11**, 455.
McKinney, G. R., Martin, S. P., Rundles, R. W. & Green, R. (1953). *J. appl. Physiol.* **5**, 335.
Malec, J. & Zakrzewski, K. (1957). *Nature, Lond.*, **180**, 551.
Marks, P. A. & Gellhorn, A. (1959). *Fed. Proc.* **18**, 281.
Marks, P. A., Johnson, A. B., Hirschberg, E. & Banks, J. (1958). *Ann. N.Y. Acad. Sci.* **75** (1), 95.
Munn, J. I. (1958). *Brit. J. Haematol.* **4**, 344.
Peters, J. P. & van Slyke, D. D. (1946). *Quantitative Clinical Chemistry, Interpretations*, vol. 1, p. 469. London: Baillière, Tindall and Cox Ltd.
Ponder, E. (1954). *Blood*, **9**, 227.
Prankerd, T. A. J. (1958). *J. Physiol.* **143**, 325.
Rowe, C. E. (1959). *Biochem. J.* **71**, 1P.
Schwartz, S., Grazia, L., Cheney, K., Hasenfus, M. B. & Cohen, S. (1956). *U.S. Atomic Energy Comm. Biological effects of external X and Y irradiation*, T.I.D. 5220, 346.
Turner, J. C. (1958). *Arch. intern. Med.* **101**, 310.
Valentine, W. N. (1956). *Progr. Haematol.* **1**, 293.
Valentine, W. N. (1958). *Meth. med. Res.* **7**, 147.
Wagner, R. & Sparaco, R. (1958). *Ann. N.Y. Acad. Sci.* **75** (1), 16.
Wintrobe, M. M. (1956). *Clinical Haematology*, 4th ed., p. 122. London: H. Kimpton.
Winzler, R. J. (1958). *Ann. N.Y. Acad. Sci.* **75** (1), 37.

Biochem. J. (1960) **75**, 320

Selective Acetylation of the Terminal Amino Group of Corticotrophin

BY J.-P. WALLER* AND H. B. F. DIXON
Department of Biochemistry, University of Cambridge

(*Received* 22 *September* 1959)

The amino acid sequence in α-melanocyte-stimulating hormone (α-MSH) is identical with that of the *N*-terminal tridecapeptide in corticotrophin (I) (Harris & Lerner, 1957; Harris, 1959). α-MSH (II) differs from the latter in having an *N*-acetyl substituent on its *N*-terminal serine, and an amide group on its *C*-terminal valine.

Ser.Tyr.Ser.Met.Glu.His.Phe Arg.Try.Gly.Lys.Pro.Val.Gly...^{39}Phe
(I)

N-Acetyl Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.(Val.NH_2)
(II)

This common sequence, part of which is also found in β-melanocyte-stimulating hormone (β-MSH), provides an explanation in terms of chemical structure for the intrinsic melanocyte-stimulating activity of corticotrophin reported by Bell (1954) and Dixon (1956*a*). However, although it contains the entire amino acid sequence of α-MSH within its structure, corticotrophin possesses melanocyte-stimulating activity of only 1% (or less) of that of α-MSH, on a weight basis.

Harris (1958, 1959) suggested that the free *N*-terminal serine in corticotrophin, which is essential for its adrenocorticotrophic activity, may also be one of the factors which inhibit the potential melanocyte-stimulating activity of the corticotrophin molecule. It was therefore of interest to determine whether selective *N*-acetylation of the *N*-terminal serine residue in corticotrophin would diminish its corticotrophic activity and also enhance its melanocyte-stimulating activity.

The *N*-terminal serine of corticotrophin cannot be selectively *N*-acetylated by the usual methods of acylation, since the ε-amino groups of the four lysine residues in the molecule would also be acetylated under the same conditions.

In their account of the synthesis of α-MSH peptide fragments, Guttmann & Boissonnas (1958) reported that treatment of the protected pentapeptide benzyloxycarbonylSer.Tyr.Ser.Met.α-methyl-γ-benzylGlu with a solution of hydrogen bromide in acetic acid, to remove the benzyloxy-

* Present address: Harvard University Biological Laboratories, Cambridge 38, Mass., U.S.A.