For the spectra of myoglobin crystals a small drop from a suspension of crystals in saturated ammonium sulphate (in H_2O) was deposited in the neck of the phial while the body contained a relatively large volume of D_2O, saturated with NaCl to bring its vapour pressure to approximately that of the crystal suspension. After 4 days at 38° C the crystals were dried by the method described above.

Insulin shows virtually complete exchange after 48 hours at 38° C. The small absorption band remaining in the N–H and O–H stretching region could be due to dilution of the D_2O with atmospheric H_2O during the preparation of the solution and addition of DCl to bring the pH to 4. After 48 hours, or longer, at 20° C a pronounced band remains at 3290 cm^{-1}. HVIDT AND LINDERSTRØM-LANG's[1,2] results for insulin also show incomplete exchange under these conditions. 3290 cm^{-1} is the main H-stretching frequency associated with the peptide bond[6], and these results are therefore consistent with the view that the less readily exchangeable protons are situated in the peptide backbone. For bovine serum albumin (Armour) after 48 hours at 38° C, or 4 days at 20° C, there is considerable but not complete exchange, and the 3290 cm^{-1} band may be further reduced if the droplet in the neck of the phial is heated to 100° C for 2 minutes before drying. The different results for insulin at 20° C and 38° C must be due to a different exchange in solution, rather than during drying, since in both cases the dry films were prepared at 20° C. Dry films formed from solutions of serum albumin which have been kept at 20° C or 38° C may be readily redissolved.

In myoglobin crystals, exposed to D_2O vapour, a band remains at 3290 cm^{-1}, and also a much less intense band at 3070 cm^{-1}. These bands show no dichroism either in the dry crystals or before drying, although there is X-ray diffraction evidence for an overall alignment of the peptide chains in seal myoglobin in the crystal form and orientation at which the spectra were taken[8]. This throws some doubt on the interpretation of the 3290 cm^{-1} band as being due to non-exchanging protons in peptide bonds. Possibly the crystals of the present sample were not identical with those on which the X-ray diffraction measurements were made.

A weak absorption band at 3070 cm^{-1} is characteristic of proteins and peptides, but has not been satisfactorily interpreted[6]. It could be due to interaction between peptide chains in the crystalline state, or to salt linkages of the type $N^+-H\cdots O^-$. In dry films prepared from myoglobin in D_2O solution the band at 3070 cm^{-1} disappears, and there is a further reduction in the absorption at 3290 cm^{-1}.

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G. H. HAGGIS

Department of Physics Applied to Medicine,
The Middlesex Hospital Medical School, London (England)

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Pathways of glucose utilization in the mammary gland of the rat

We have shown previously[1] that levels of activity of enzymes of the hexose monophosphate oxidative pathway of glucose metabolism in the rat mammary gland vary considerably according to the physiological state of activity of the gland. It was found that levels of activity of both glucose 6-phosphate (G 6-P) dehydrogenase and 6-phosphogluconate (6-PG) dehydrogenase increase rapidly from the end of pregnancy to the end of lactation, these increases being approximately 60 fold for G 6-P dehydrogenase and 20 fold for 6-PG dehydrogenase, and then fall to

very low values in the involuting gland. In an attempt to substantiate these findings and to assess the relative importance of glycolytic and non-glycolytic pathways of carbohydrate metabolism, the utilization of glucose 1-^{14}C (G 1-^{14}C) and glucose 6-^{14}C (G 6-^{14}C) by rat mammary gland slices has been determined by measuring the conversion of these specifically labelled substrates to $^{14}CO_2$. In these experiments, 500 mg mammary gland slices approximately 0.4 mm in thickness, prepared with a Stadie-Riggs cutter, were introduced into special flasks[2] containing 4.5 ml Krebs-Ringer bicarbonate solution and 0.2 ml glucose solution containing approximately 0.2 μc ^{14}C in 20 mg of glucose. The side arm contained 0.5 ml 5 *N* H_2SO_4. After incubation for 1½ hours at 38° C with 5% CO_2/95% O_2 as the gas phase, 1 ml 5 *N* KOH was introduced into the centre well by injection through the rubber teat and the H_2SO_4 tipped in from the side arm. A further 60 minutes was allowed before the contents of the centre well were transferred and diluted with water to 10 ml Aliquots of this solution were used for the determination of $^{14}CO_2$ by gas counting[3]. The results for the percentage conversion of G 1-^{14}C and G 6-^{14}C to $^{14}CO_2$ are represented graphically in Fig. 1. The preferential utilization of G 1-^{14}C throughout lactation indicates the active participation of a non-glycolytic route, average values for the yield of $^{14}CO_2$ from G 1-^{14}C/yield of $^{14}CO_2$ from G 6-^{14}C being 1.1 at the end of pregnancy 15.7 between the 10th and 18th day of lactation and 2.1 at the beginning of involution. These isotope data, however, indicate maximum activity of an alternative non-glycolytic pathway between the 10th and 18th of lactation whereas the G 6-P and 6-PG dehydrogenase activities increase steadily to the end of lactation. This might indicate the existence of yet another alternative pathway, such as the glucose dehydrogenase system, which would also preferentially oxidise G 1-^{14}C. Our data for the middle of lactation agree well with those of ABRAHAM, HIRSCH AND CHAIKOFF[4] who not only used G 1-^{14}C but in addition glucose labelled with ^{14}C in other positions and also three ^{14}C labelled lactates. These workers, moreover, who restricted their observations to the rat mammary gland in the middle of lactation, obtained additional support for the existence of a non-glycolytic pathway since more ^{14}C labelled long chain fatty acids were recovered from G 6-^{14}C than from G 1-^{14}C.

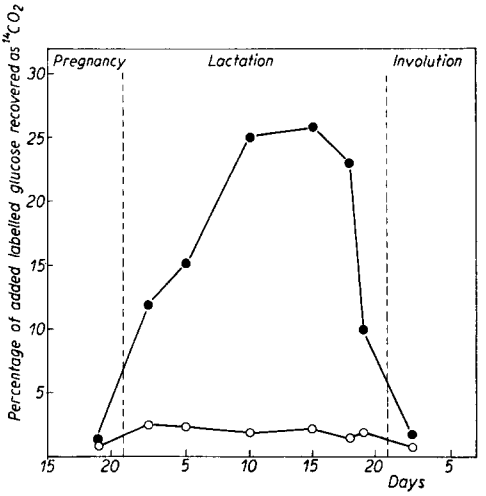

Fig. 1. The relative amounts of $^{14}CO_2$ formed from glucose 1-^{14}C and glucose 6-^{14}C by rat mammary gland slices. ●—● $^{14}CO_2$ formed from glucose 1-^{14}C; ○—○ $^{14}CO_2$ formed from glucose 6-^{14}C.

The significance of this striking increase in the activity of the hexose monophosphate oxidative pathway during lactation is at present obscure. It is not merely a reflection of a general increase in protein synthesis since the data with G 6-^{14}C indicate that the activity, at least of the glycolytic pathway, remains relatively constant throughout lactation.

It would be of interest to extend these experiments to mammary gland slices of ruminants since, in contrast to the rat, these utilise acetate much more readily than glucose for fatty acid synthesis[5]. Moreover, we have found that the G 6-P and 6-PG dehydrogenase activities of the sheep mammary gland are increased less than twofold during lactation, which is in striking contrast to the results obtained in the rat.

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Courtauld Institute of Biochemistry and Barnato-Joel Laboratories, Middlesex Hospital, London (England)

G. E. GLOCK
P. MCLEAN
J. K. WHITEHEAD

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