It was suggested to us by Dr R. B. Loftfield that the most likely explanation for the excess of labelling in the presence of L-[^{14}C]leucine is the presence of small quantities of other amino acids. This point does, however, need further examination.

The importance of cell-sap RNA in the specific selection of amino acids before their incorporation into the peptide-chain sequence is further emphasized by our experiments with glutamic acid and glutamine. It has been shown by Sansom & Barry (1958) that asparagine and glutamine are incorporated into milk protein independently from aspartic acid and glutamic acid respectively. We were able to show that the binding sites on cell-sap RNA can distinguish between glutamine and glutamic acid. Though the instability of glutamine made it impossible to obtain quantitative information for this amino acid it seems likely that the cell-sap RNA-coupling process is the point in protein synthesis at which the distinction occurs between the dicarboxylic amino acids and their amides.

SUMMARY

1. Systems were developed for the study of the reactions of the enzyme systems and cell-sap ribonucleic acid of guinea-pig mammary tissues with [^{14}C]amino acids and adenosine triphosphate.

2. It was shown that the quantities of amino acids taken up by such systems depend on the amino acid concentration and on the cell-sap ribonucleic acid concentration.

3. Though it is clear that there are specific sites for each amino acid studied, the precise number of sites is difficult to evaluate because of non-specific reactions.

4. From the data obtained the conclusion can be drawn that the extent of combination of amino acids with cell-sap ribonucleic acid determines the quantities of each amino acid to be incorporated into protein. This step may also be responsible for conferring additional specificity on amino acids to determine their addition to polypeptide chains in the correct position in the sequence.

We are grateful to the Rockefeller Foundation for financial support for the purchase of equipment for the Physiology Department, and for the award of a Fellowship to one of us (H.S.). We wish to express our thanks to Dr S. J. Folley, F.R.S., who instigated this programme of research on the biosynthesis of protein in the mammary gland, for his encouragement, to Dr M. Hoagland for much useful advice and to Mrs Margaret Bond for skilful technical assistance.

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Turnover of Nucleic Acids in a Non-Multiplying Animal Cell

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Turnover of nucleic acids may be defined as the continuous renewal of these compounds within the cell by a balanced process of degradation and resynthesis. The idea that ribonucleic acids turn over in this way is derived from experiments on animals (Smellie, 1955). But the complexity of animal experiments does not lend itself to measurement of the rate of intracellular turnover (Cohn, 1957), and the results which have been obtained in such experiments are difficult to interpret. Animal experiments have failed to decide whether turnover of the deoxyribonucleic acids occurs at all. There appear to have been only two studies of nucleic acid turnover in animal cells cultivated *in vitro*. In the first of these (Siminovitch & Graham, 1956) cells in exponential growth were studied. The retention of radioactive phosphorus in the nucleic acids was used to measure turnover, but no turnover was detected either in ribonucleic acid or in deoxyribonucleic acid. In the second study

(Thomson, Paul & Davidson, 1958) the retention of radioactive bases in the nucleic acids was used to measure turnover, and the results appeared to indicate that there was a slow turnover of ribonucleic acid, but no turnover of deoxyribonucleic acid. In this study, however, the growth kinetics of the cell cultures were not measured and repeated subcultures involving traumatic procedures were made during the course of the experiments.

The direct measurement of turnover in nucleic acids entails measurement of the extent to which a radioactively labelled compound which has been incorporated into the nucleic acids is retained in them. However, if turnover occurs, it is possible that the cell may utilize the products of turnover again for the synthesis of new nucleic acids; that is, continuous recycling of these products may take place. If these products are completely utilized, a labelled compound which has been incorporated into the nucleic acids may be completely retained in them. Turnover will not then be detected unless the medium contains some unlabelled compound which has ready access to the cell and which is able to displace or dilute a labelled intermediate in the process of recycling. The nature of the products of nucleic acid turnover, and hence the choice of unlabelled exogenous compounds which might be expected to dilute labelled products, do not appear to have received much attention. In the experiments of Siminovitch & Graham (1956) the nucleic acids were labelled with radioactive phosphorus, and unlabelled orthophosphate was used to dilute any labelled intermediates of turnover which might have been formed; and in the experiments of Thomson *et al.* (1958) the nucleic acids were labelled with radioactive formate, and unlabelled formate was used to dilute the labelled intermediates. But there is no reason to assume that the products of nucleic acid turnover include either orthophosphate or formate; a finding of little or no turnover under these conditions might mean no more than that orthophosphate and formate do not effectively dilute any labelled intermediate.

The experiments described in this paper were carried out on the macrophages of the rabbit. Virtually pure populations of these cells are easily obtained from the peritoneal cavity of the rabbit. The cells remain alive and motile *in vitro* for at least 12 hr., but they do not multiply or grow. They are therefore well suited to studies on turnover, since no corrections need be made for changes in the size of the cell population or in the amount of nucleic acid per cell.

EXPERIMENTAL

Preparation of macrophage cultures. The macrophages were obtained from the peritoneal cavity of the rabbit some days after intraperitoneal injection of liquid paraffin (Lucké, Strumia, Mudd, McCutcheon & Mudd, 1933). The exudates from three rabbits were spun at 220 *g* for 3 min. and the cells were resuspended in 150–200 ml. of medium consisting of 10% of rabbit serum in Hanks's (1948) solution. Measured volumes (10 ml.) of this suspension were introduced into Carrel flasks of 85 mm. diameter. The flasks were incubated at 37° for 40 min. to allow the macrophages to settle and stick to the floor of the flask. The medium was then withdrawn and the adherent layer of cells washed vigorously with physiological saline (0·85% NaCl soln.). Erythrocytes, lymphocytes and dead cells do not adhere to glass, and polymorphonuclear leucocytes adhere poorly. Washing the adherent layer of cells thus removes all but living macrophages and a few cells of fibroblast morphology.

Culture media and radioactive compounds. In the experiments in which the incorporation of radioactive adenine was studied, the cells were maintained in a medium consisting of 25% (v/v) of rabbit serum, which had been dialysed against 0·85% NaCl soln., in Eagle's basal medium (Eagle, Oyama, Levy, Horton & Fleischman, 1956). [8-^{14}C]Adenine was added to make a final concentration of 0·2 mM (0·5–1·0 μc/ml. of medium). In the experiments with radioactive phosphorus the same medium was used, but unlabelled phosphate was omitted. [^{32}P]Orthophosphate was added to make a final concentration of 0·5 μc/ml. of medium. The rabbit serum contained a phosphatase active against adenosine 3′-phosphate and an adenosine deaminase, both of which remained active after dialysis. In experiments involving the use of nucleotides and nucleosides therefore the medium consisted of 20% (v/v) of a 5% (w/v) solution of crystalline human serum albumin in Eagle's medium. The albumin showed no phosphatase or deaminase activity.

When retention of labelled compounds in the nucleic acids was studied the cells were first incubated for 4 hr. at 37° in medium containing the radioactive compound. This medium was then decanted and the cells were washed with a large volume of 0·85% NaCl soln. They were then incubated for 8 hr. in medium containing no radioactive compounds. The various unlabelled purines and purine derivatives were added to this medium at a concentration of 0·2 mM. Eagle's basal medium contains mM-orthophosphate; no extra phosphate was added to this when retention of labelled phosphorus was studied.

Preparation of nucleic acid fractions. The cells were washed three times with 0·85% NaCl soln. and the contents of each flask were harvested in 3 ml. of water containing 1% (v/v) of the detergent Teepol (Shell Chemicals Ltd.); this removed the cells from the glass and completely disrupted them. The solution containing the disrupted cells was made 0·3 N with respect to trichloroacetic acid and the resulting precipitate was spun down; these operations were carried out at 0°. The supernatant was discarded and the precipitate was resuspended in water and spun down again. Lipids were extracted from the precipitate with ethanol at 45° for 30 min. followed by ethanol–ether (1:1) at 45° for 30 min. The residue was hydrolysed with 1 ml. of 0·3 N-KOH for 18 hr. at 37°. 10 N-Perchloric acid was then added at 0° until the pH was 1–2 and the resulting precipitate was spun down at 0°. The supernatant was the ribonucleic acid (RNA) fraction. The precipitate was dissolved in 0·3 N-KOH and reprecipitated by acidification with trichloroacetic acid at 0°; it was then washed with

water and extracted with 0·3 ml. of 0·3N-trichloroacetic acid at 90° for 30 min. This extract was the deoxyribonucleic acid (DNA) fraction.

Isolation of nucleotides and purine bases. When the phosphorus of the RNA fraction was examined the nucleotides in this fraction were separated by electrophoresis on Whatman no. 1 filter paper in 0·05M-ammonium formate buffer, pH 3·5; 4 hr. at 13 v/cm. in the apparatus described by Flynn & de Mayo (1951) gave adequate resolution. In other experiments the RNA fraction was hydrolysed with N-HCl at 100° for 1 hr.; this yielded a mixture of purine bases and pyridimidine nucleotides (Loring, 1955). The hydrolysate was evaporated to dryness *in vacuo*. This procedure was altered when it was found that [8-^{14}C]-adenine contributed no radioactivity to the pyrimidine bases. The RNA fraction was then hydrolysed with N-H_2SO_4 instead of N-HCl, and the silver salts of the purines were precipitated (Loring, 1955). The purines were extracted from the silver salts with 0·1N-HCl and the extract was evaporated to dryness *in vacuo*. The residues were dissolved in 95% ethanol, and applied to sheets of Whatman no. 1 filter paper for chromatography, which was carried out with a descending-solvent system composed of methanol–ethanol–water–conc. HCl (50:25:19:6, by vol.) (Kirby, 1955). The purines of the DNA fraction were also isolated by means of their silver salts and subjected to chromatography in the same system.

Measurements on the culture medium. When the radioactive compounds appearing in the medium were investigated a single flask containing as many cells as would adhere to the floor was used. The cells were incubated in the presence of [8-^{14}C]adenine for 4 hr.; the radioactive medium was removed and the cells were washed once with 0·85% NaCl soln. containing an excess of unlabelled adenine and three times with saline alone. Non-radioactive medium (15 ml.) was then introduced into the flask and incubation continued. Samples (0·5 ml.) of this medium were withdrawn at regular intervals. Each sample of medium was deproteinized with 0·3N-trichloroacetic acid at 0° and the supernatant was hydrolysed with 0·1N-H_2SO_4 for 1 hr. at 100°. This hydrolysis gave adequate recoveries. The purines were isolated by precipitation of their silver salts and subjected to chromatography, first in the solvent system described above, which resolved adenine from guanine, and then in the same dimension in butanol–water–ammonia (MacNutt, 1952), which resolved adenine from hypoxanthine. The paper used in these experiments was eluted before use, first with 0·5N-HCl for 2 days and then with water for 2 days, to remove the ultraviolet-absorbing material which accumulated at the solvent front in the acid system.

The medium remaining at the end of the experiment was deproteinized at 0° with 0·3N-perchloric acid. After the precipitate had been spun down the supernatant was collected and adjusted to pH 4–5 with 5N-KOH. The solution was then run through a small column of charcoal (Nuchar C, West Virginia Pulp and Paper Co., N.Y., U.S.A.) and the adsorbed material was eluted with 15 ml. of aq. 40% (v/v) ethanol containing 1% of aq. NH_3 soln. (sp.gr. 0·880) (Cabib, Leloir & Cardini, 1953). The eluate was evaporated to dryness *in vacuo* and subjected to chromatography in the two solvent systems described above.

Determination of specific activity. The radioactive regions on the chromatograms were located by radioautography on X-ray film (Kodak Ltd., Blue Brand). The films were exposed for 3 weeks. Ultraviolet-absorbing regions were located by the method of Holiday & Johnson (1949). These were cut out and eluted overnight into 0·5 ml. of 0·01N-HCl. The ultraviolet-absorption spectra of the eluates were measured in 1 cm. micro cells in a Hilger Uvispek spectrophotometer. Known volumes of the eluates were dried on glass coverslips and the radioactivity was measured with a mica end-window Geiger–Müller tube (type EHM2S, General Electric Co.).

Radioautographic examination of fixed cell preparations. A monolayer of macrophages was allowed to adhere to a glass slide, and the adherent cells were washed with 0·85% NaCl soln. in the usual way. They were then incubated for 8 hr. at 37° in medium containing tritium-labelled thymidine (Schwarz Laboratories Inc., N.Y., U.S.A.) (0·5 μc/ml. of medium). The specific activity of the thymidine was 360 mc/m-mole. After incubation the preparation was washed with saline and fixed in methanol. The cells were extracted with ice-cold 0·3N-trichloroacetic acid, washed and dried. Radioautographs were prepared by the stripping-film technique, with Kodak AR. 10 film (Pelc, 1956). The films were exposed for 1 week and then developed. The cells were stained through the film with nuclear-fast red.

Measurement of changes in the ratios of ribonucleic acid and protein to deoxyribonucleic acid. The macrophages were maintained in flasks in medium consisting of 25% of dialysed rabbit serum in Eagle's medium. The cells in one group of six flasks were harvested after incubation for 1 hr. and those in another group of six flasks after incubation for 12 hr. The procedure described for the preparation of nucleic acid fractions was carried out to the stage where the RNA fraction was removed from the precipitate of DNA and protein. The precipitate was dissolved in KOH soln. at pH 12 and the extinction of the solution was measured at 240 and 260 mμ. At 240 mμ the specific extinction of protein is high relative to that of DNA, whereas at 260 mμ the specific extinction of DNA is high relative to that of protein. Change of the ratio of extinctions at these wavelengths therefore provides a measure of any change of the proportion of DNA to protein. Model experiments with mixtures of human serum albumin and purified DNA showed that a 5% change in the proportion of DNA to protein could be detected.

RESULTS

Amount of ribonucleic acid and protein per cell. Over a period of 12 hr. no change in the ratio of RNA to DNA or of protein to DNA could be detected. Since other experiments, which are described below, showed that no synthesis of DNA occurred in the macrophage, it was concluded that the amount of RNA and protein per cell remained constant during the course of the experiments.

Incorporation of [8-^{14}C]*adenine into nucleic acids.* Radioactive adenine, rather than adenosine, was chosen as the precursor of the nucleic acid purines because exogenous adenosine was rapidly deaminated both by the rabbit serum and by the macrophages. Adenine was not deaminated under

the same conditions. When the cells were incubated with [8-^{14}C]adenine, both the adenine and the guanine of the nucleic acids became radioactive. No activity was detected in the protein fraction of the cells.

(*a*) Deoxyribonucleic acid. Macrophages which had been incubated in the presence of tritiated thymidine showed no radioactive labelling in radioautographs. A few cells of fibroblast morphology, which represented less than 1 % of the total cell population, showed heavy nuclear labelling. These cells are known to multiply. Macrophages which had been incubated with [8-^{14}C]adenine were fractionated by the procedure described above for the preparation of nucleic acid fractions. The specific activity of the adenine in the DNA fraction was less than 1 % of the specific activity of the adenine in the RNA fraction. The trace of radioactivity in the adenine of the DNA fraction could be explained by the presence of the few multiplying cells. Since neither adenine nor thymidine was incorporated into the DNA of the macrophages it can be concluded that there was no synthesis of DNA and no turnover of adenine or thymidine residues.

(*b*) Ribonucleic acid. The incorporation of [8-^{14}C]adenine into the purines of the RNA fraction is shown in Fig. 1. The rate of incorporation varied somewhat from experiment to experiment; usually about 20 % of the adenine in the RNA was labelled at the end of 12 hr.

The incorporation of adenine was sensitive to azide: a concentration of 5 mM-sodium azide in the medium caused a 35 % reduction in the amount of labelled adenine incorporated into RNA in 8 hr. The specific activity of the guanine in the RNA was reduced by a similar proportion.

Retention of labelled adenine in ribonucleic acid. When cells which had been labelled by incubation for 4 hr. with [8-^{14}C]adenine were transferred to medium which contained no added purines or purine derivatives none of the labelled adenine was displaced from the RNA during a period of 8 hr. If, however, adenine or certain purine derivatives were added to the medium, labelled adenine was displaced from the RNA (Fig. 2). In the presence of adenine, inosine or the 2′-, 3′- and 5′-phosphate of adenosine, the fall in specific activity was about 20 % in 8 hr. In the presence of inosine 5′-phosphate no fall in the specific activity occurred during a period of 8 hr. Adenosine, however, produced a

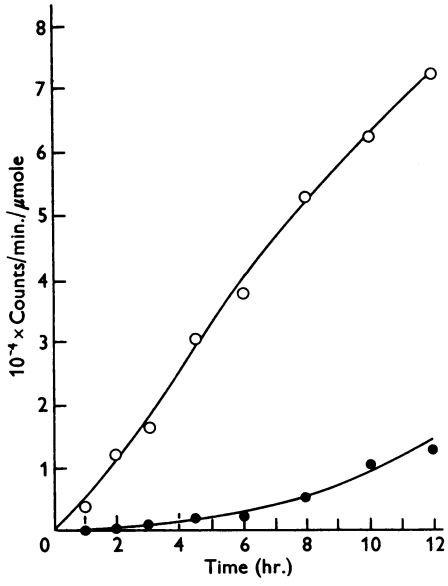

Fig. 1. Incorporation of [8-^{14}C]adenine into the purines of the RNA of macrophages. Macrophages were maintained in medium containing 0·2 mM-[8-^{14}C]adenine for 12 hr. Samples of the cells were harvested at intervals and the specific activities of the adenine and guanine in the RNA were determined. ○, RNA adenine; ●, RNA guanine.

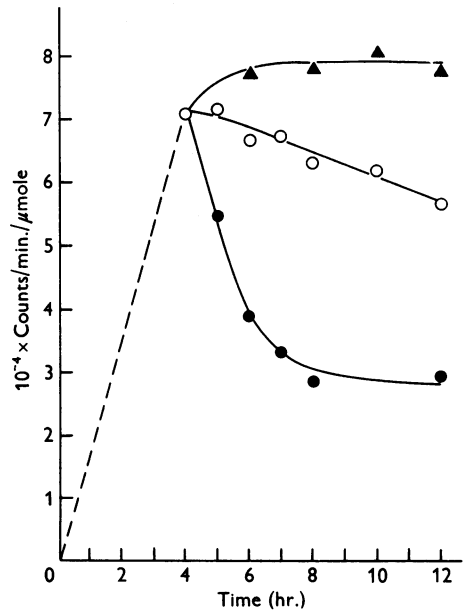

Fig. 2. Retention of radioactive adenine in the RNA of macrophages. The adenine of the RNA was labelled by incubating the cells for 4 hr. in the presence of 0·2 mM-[8-^{14}C]adenine. The cells were then transferred to non-radioactive medium which contained the purine derivative at a concentration of 0·2 mM. Samples of the cells were harvested at intervals and the specific activity of the adenine in the RNA was determined. ▲, Inosine 5′-phosphate in the medium; ○, inosine in the medium; ●, adenosine in the medium.

dramatic effect: in the presence of this compound the specific activity fell by 50% in about 2 hr. The kinetics of the fall in specific activity in the presence of adenosine are complicated by the fact that the cells rapidly convert exogenous adenosine into inosine. Under the present conditions all the adenosine had disappeared from the medium in about 6 hr. so that during the latter part of the experiment the medium contained mainly inosine, which displaced the labelled adenine from RNA less efficiently than did adenosine.

The fall in the specific activity of the RNA adenine in the presence of exogenous purine and purine derivatives was also azide-sensitive. In the presence of exogenous adenine this fall was about as sensitive to 5 mM-sodium azide as the incorporation of [8-^{14}C]adenine into RNA: the fall in specific activity was reduced by 35% in 8 hr. In the presence of exogenous adenosine, however, 5 mM-sodium azide reduced the fall in specific activity by only 6–8% over the same period. This suggests that the incorporation of adenine into RNA involves azide-sensitive processes which are not involved in the incorporation of adenosine.

Appearance of radioactive compounds in the medium. The fall in the specific activity of the RNA adenine was accompanied by the appearance of radioactive compounds in the medium. The increase in the radioactivity of the medium was proportional to the fall in the specific activity of the RNA. However, whatever compound was used to displace the labelled bases from the RNA, the main radioactive compound accumulating in the medium was inosine. Some activity also appeared in a fraction which has been tentatively identified by paper chromatography as guanosine.

The accumulation of radioactive inosine in the medium when labelled cells were incubated in the presence of unlabelled adenosine is shown in Fig. 3. The upper curve shows the specific activity of the inosine fraction; this falls progressively mainly because of the rapid production of unlabelled inosine in the medium by deamination of exogenous adenosine. The lower curve shows the radioactivity

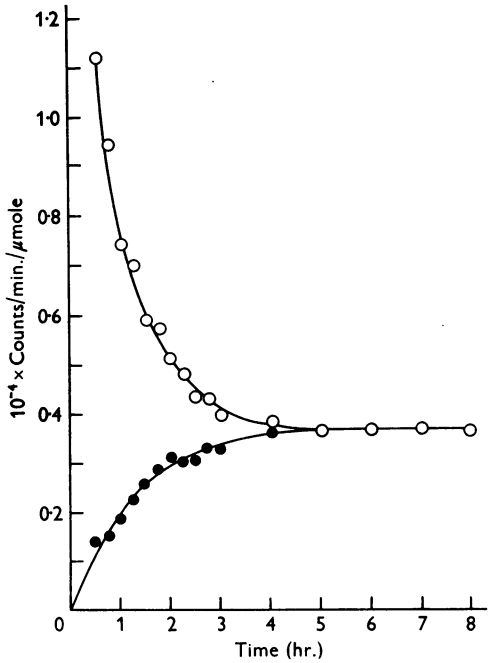

Fig. 3. Accumulation of radioactive inosine in the medium when macrophages in which the RNA had previously been labelled were incubated in non-radioactive medium. The adenine in the RNA had been labelled by incubating the cells for 4 hr. in the presence of [8-^{14}C]adenine. The non-radioactive medium contained 0·4 mM-adenosine. Samples of the medium were taken at intervals and the specific activities of inosine and adenosine were determined. ○, Specific activity of inosine; ●, specific activity of (adenosine + inosine).

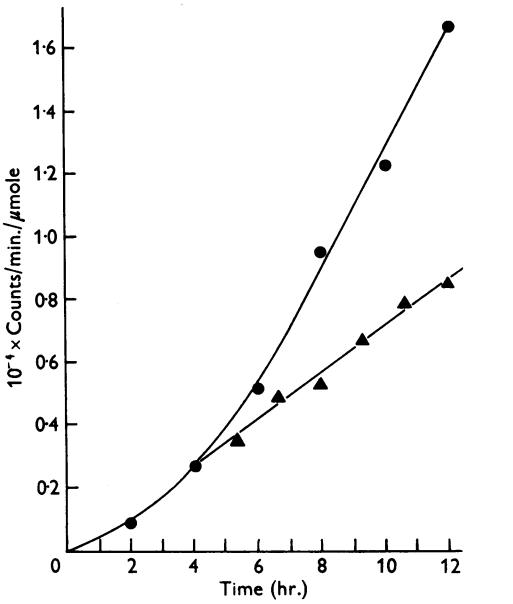

Fig. 4. Incorporation and retention of [^{32}P]phosphate in the cytidylic acid of the RNA. Macrophages were incubated in the presence of [^{32}P]orthophosphate for periods up to 12 hr. Samples were taken at intervals and the specific activity of the cytidylic acid was determined (●). Six flasks were incubated for 4 hr. in the radioactive medium and then transferred to non-radioactive medium containing unlabelled orthophosphate. Samples were again taken at intervals and the specific activity of the cytidylic acid was determined (▲).

in the inosine fraction relative to the total amount of inosine + adenosine present in the medium, thus allowing for the process of deamination. The specific activity of the purines in the medium reached half their maximum value in about 55 min. An apparent equilibrium was reached at the same time as the deamination of exogenous adenosine was complete.

Incorporation and retention of labelled phosphorus in ribonucleic acid. The incorporation of [^{32}P]-phosphate and its retention by the cells were measured under the same conditions as those used to measure the incorporation and retention of [8-^{14}C]adenine. The nucleotides of the RNA fraction were resolved and their specific activities were determined. Fig. 4 illustrates the incorporation and retention of ^{32}P in cytidylic acid; the results obtained with the other nucleotides were similar. It will be seen that the specific activity of the nucleotide continued to rise after the cells had been transferred from the radioactive medium to non-radioactive medium, despite the fact that any residual [^{32}P]orthophosphate was diluted at least 100-fold by the unlabelled orthophosphate of the non-radioactive medium. It thus appears that the labelled intermediates involved in the turnover of RNA are not effectively diluted by unlabelled orthophosphate over the period studied.

DISCUSSION

Since the amount of RNA in the cell remains constant, the incorporation of labelled adenine into RNA and its displacement by certain exogenous purines and purine derivatives indicate that at least some of the RNA is continuously degraded and resynthesized. The half-life of the RNA involved in this turnover is less than 2 hr. The kinetics of the retention of labelled adenine in RNA show that the cell utilizes again the degradation products of RNA turnover with great efficiency (Fig. 2). The observed rate of turnover is therefore determined by the ease with which an unlabelled exogenous compound can enter the pathway by which the degradation products are recycled and so displace or dilute a labelled intermediate. The importance of the choice of the compound to be used for this purpose is shown by the fact that with two of the compounds tested in the present experiments, inosine 5′-phosphate and orthophosphate, no turnover of RNA could be demonstrated over the period studied. With adenine, the 2′-, 3′- and 5′-phosphate of adenosine and inosine, the observed rates of turnover were slower than with adenosine, thus indicating that these compounds had a more restricted access to the turnover pathway than adenosine. But even with adenosine the observed rate of turnover can be regarded only as a minimum value, for it, too, may be limited by the rate at which a labelled intermediate is diluted rather than the rate at which it is recycled. The true rate of turnover remains unknown.

SUMMARY

1. Turnover of ribonucleic acid and deoxyribonucleic acid was studied in rabbit macrophages. These cells remain alive and motile *in vitro* for long periods but do not multiply. The amounts of ribonucleic acid and protein per cell remain constant.

2. No synthesis of deoxyribonucleic acid and no turnover of deoxyribonucleic acid adenine or thymidine residues could be detected.

3. As measured by the incorporation and release of [8-^{14}C]adenine, a rapid turnover of ribonucleic acid occurred. It could be shown, however, that the breakdown products of this turnover were reincorporated into ribonucleic acid with great efficiency.

4. The observed rate of turnover was thus determined by the ease with which an unlabelled exogenous compound could enter the pathway by which the products of turnover were recycled and so displace or dilute a labelled intermediate. In this respect adenine, the 2′-, 3′- and 5′-phosphate of adenosine and inosine were all less effective than adenosine. In the presence of exogenous adenosine the observed half-life of the ribonucleic acid involved in turnover was less than 2 hr.; with the other compounds mentioned the observed half-life was about 12 hr. With inosine 5′-phosphate no turnover could be detected.

5. As labelled adenine was displaced from the ribonucleic acid, a comparable amount of labelled inosine appeared in the culture medium.

6. ^{32}P was incorporated into ribonucleic acid but could not be displaced from it by unlabelled orthophosphate over the period studied.

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A Lipophosphoprotein Complex in Hen Plasma Associated with Yolk Production

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The blood of the laying hen is characterized by a very high lipid content, especially of triglyceride (e.g. Lorenz, Entenman & Chaikoff, 1938; Walker, Taylor & Russell, 1951). It is also characterized by the presence of protein phosphorus ('serum vitellin') demonstrated by Laskowski (1935*a*) and Roepke & Hughes (1935), who observed its virtual absence from the serum of non-laying birds. The high lipid and phosphoprotein content of laying-hen plasma is apparently the result of high oestrogen production (see Lorenz, 1954, and Sturkie, 1954, for reviews).

Laskowski (1935*b*) obtained a phosphoprotein fraction as a precipitate by dilution of laying-hen plasma, and Roepke & Bushnell (1936) observed the precipitation of phosphoprotein on dialysis of hen serum. Both groups of workers, although apparently recognizing the presence of lipid in the precipitable material, made no study of it. More recently, McKinley, Oliver, Maw & Common (1953) and McKinley, Maw, Oliver & Common (1954) presented evidence for the occurrence of phosphoprotein in the material precipitated from the serum of pullets treated with oestrogens, on dilution.

This paper deals with the conditions of preparation of the precipitable lipophosphoprotein from laying-hen plasma, and observations on its composition and properties. In addition its behaviour during the pre- and post-laying periods has been studied. A preliminary account of some of this work has been given (McIndoe, 1957).

METHODS

Animals. Brown Leghorn hens (2 years old), fed on the Poultry Research Centre diet (Bolton, 1958), were used. They were kept in battery cages under artificial lighting. When it was desired to put the hens off lay the amount of light they received was reduced from 14 to 6 hr./day by 1 hr./day (0·5 hr. at either end). To bring them into production this procedure was reversed.

Preparation of precipitable lipophosphoprotein. Blood (usually 3–5 ml.) was taken by syringe from the wing vein and added to a small amount of sodium citrate (about 0·5 mg./ml. of blood). Plasma was diluted with 9 vol. of water, allowed to stand in the refrigerator for 1½–2 hr. and centrifuged at 3000*g* for 20–30 min. at 2–5°. The supernatant fraction was retained. The precipitate was dissolved in water and reprecipitated by bringing the NaCl concentration to 0·015M. After standing at 2° for 15 min. the precipitate (PLP) was centrifuged as before for at least 40 min.

Extraction of lipids. Absolute ethanol (20 ml.) was added to the precipitate from 2 ml. of plasma, mixed and allowed to stand overnight. The protein was subsequently extracted with 20 ml. of ethanol–ether (3:1, v/v) three times and finally with ether. The extracts were combined, reduced to less than 10 ml. on a steam bath and evaporated to dryness in a vacuum desiccator over P_2O_5. The supernatant fraction was mixed with 4 vol. of ethanol and the lipids were extracted from the precipitated protein as described above. To remove water-soluble contaminants, water and ether were added to the combined aqueous ethanol–ether extract until an aqueous phase developed. The organic phase was evaporated to dryness as above. Before analysis the dried lipids were dissolved in dry $CHCl_3$.

Analysis of lipids. Total lipid was estimated gravimetrically. (Values for the supernatant fraction are approximate, owing to the relatively low lipid content.) Subsequently the phospholipid (P × 25) was determined by phosphorus analysis (Allen, 1940) and cholesterol by the Liebermann–Burchard reaction (Hawk, Oser & Summerson, 1947). The error in the latter estimation, due to the possible non-equivalent colour intensities of cholesterol and cholesterol esters, is likely to be small since only about 20% of hen-plasma cholesterol is esterified (Walker *et al.* 1951). In a few cases free and total cholesterol were determined by the above-mentioned colorimetric method after using the saponification and digitonin-precipitation procedures of Schoenheimer & Sperry (1934).

Protein. Samples of protein for the determination of N and P were dried at 110° for 18 hr. and allowed to cool in a