ADDENDUM

Preparation of Interferon

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The method used was basically that described by Burke & Isaacs (1958) and consisted in exposing chick chorio-allantoic membranes to influenza virus (Melbourne), irradiated by ultraviolet light, and harvesting the culture fluid.

The inactivated seed virus was prepared by irradiating 3 ml. of allantoic fluid containing the Melbourne strain of influenza virus in a 4 in. Petri dish for 1 min., 7 cm. beneath a Phillips T.U.V. tube with a maximum emission at 2537Å. The haemagglutinating capacity of the inactivated-virus preparation was determined by making serial twofold dilutions of this material in 0·9% NaCl soln. in 0·25 ml. volumes and adding an equal volume of 0·5% fowl red-cell suspension to each dilution. After settling at room temperature the sedimentation patterns were observed. One agglutinating unit was taken as the highest dilution giving partial agglutination, and the titres were expressed as the reciprocal of the initial dilutions of inactivated virus at the end point. The seeding of the 5 l. glass bottles containing the membranes was at the rate of 1000 haemagglutinating units per membrane.

Eggs were incubated in large commercial incubators for 10 days, and the fertile eggs, after swabbing with 50% aqueous ethanol, were drilled through the end opposite the air sac. After removal of this part of the shell, the embryo, yolk sac and allantoic fluid were pulled out and discarded. The chorio-allantoic membranes were removed and placed in a beaker of Earle's medium (40 membranes per beaker). The Earle's medium at all stages was reinforced with 500 μg. of benzylpenicillin/ml. and 500 μg. of streptomycin sulphate/ml.

Each membrane was washed individually in more Earle's medium to clear it of yolk, albumin and blood, and 40 washed membranes were placed in a 5 l. glass bottle containing 200 ml. of medium. These bottles were seeded with ultraviolet-irradiated Melbourne virus and rocked in a 37° incubator for 3 hr.

After incubation for 3 hr. with seed virus, the membranes were removed from the bottles, washed in Earle's medium to remove surplus virus and put into further 5 l. bottles, each containing 200 ml. of Earle's medium. They were then rocked at 37° for 16–18 hr., when the first harvest was taken. The membranes were reincubated as before in Earle's solution in fresh 5 l. bottles for a second harvest.

The culture fluid containing interferon was harvested in 500 ml. blood bottles and clarified by centrifuging at 320 g for 20 min. The supernatant interferon solution was removed and tested for sterility. The second harvest received identical treatment.

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Lipo-amino Acid Complexes from *Bacillus megaterium* and their Possible Role in Protein Synthesis

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In the course of studies of protein synthesis in cell-free systems derived from *Bacillus megaterium*, it was found that some of the labelled amino acids apparently incorporated into protein could be solubilized by methanol, lipase or dilute alkali and obtained in a form no longer precipitable by trichloroacetic acid (Hunter, Brookes, Crathorn & Butler, 1959). When the labelled membrane fraction was extracted with deoxycholate, it was further found that the solubilized material contained protein having a higher specific radioactivity than the protein of the insoluble portion. These results stimulated a study of the possible role of lipids in the amino acid incorporation process, and it has

been shown that lipoidal substances become labelled very rapidly when [^{14}C]amino acids are incubated with protoplasts or the cytoplasmic membrane fraction of *B. megaterium*. As briefly reported elsewhere (Hunter & Goodsall, 1960) these lipo-amino acid complexes possess many of the properties to be expected of precursors of protein, and a more detailed account of the work is now given.

METHODS

Organism. The strain of *B. megaterium* (originally KM) was cultured as described by Butler, Crathorn & Hunter (1958). For all the experiments described here the organism was grown at 30° in a glucose–salts medium (C medium, McQuillen, 1955).

Experiments with protoplasts. As described by Hunter *et al.* (1959), the conversion of whole cells (approx. 4 mg./ml.) into protoplasts was effected at 30° in a medium containing 0·02% of lysozyme and 0·5 M-KH$_2$PO$_4$, brought to pH 7·0 with NaOH. The protoplasts were sedimented and then resuspended (approx. 3 mg./ml.) in a medium of the following composition (%, w/v), finally brought to pH 7·0 with NaOH: glucose, 1; NH$_4$Cl, 0·2; Na$_2$HPO$_4$, 0·6; KH$_2$PO$_4$, 7·15; NaCl, 0·3; Na$_2$SO$_4$,10H$_2$O, 0·025; MgCl$_2$,6H$_2$O, 0·0085. The protoplast suspensions were aerated by rotary shaking at 30° for 30–60 min. before the labelled amino acids were added, and the shaking was then continued for the time required. The incubations were usually terminated by cooling the incubation flask and its contents as rapidly as possible to about 5° and then the protoplasts were sedimented in a centrifuge precooled to 0–5°. Chloramphenicol and crotoxin (phospholipase A, kindly supplied by Dr C. Long) were both used at concentrations of 200 μg./ml. Extractions of the protoplasts with acetone or methanol were usually carried out at 0–5° after removal of the culture medium by centrifuging; approx. 100 ml. of solvent was used for each gram of protoplasts. Occasionally the extractions were carried out immediately, when the organic solvent was added in a final concn. of 90% (v/v) to the whole incubation medium. The clear extracts obtained by centrifuging were freeze-dried below 50°. The centrifugal sediment still contained the ribonucleic acid (RNA) which could be quantitatively extracted by the phenol–ethylenediaminetetra-acetate method (Littauer & Eisenberg, 1959).

Paper chromatography and radioautography. The routine paper-chromatographic separation of lipo-amino acid complexes from amino acids was carried out by upward flow on Whatman 3 MM paper with butan-2-one–acetic acid–water (3:1:1, by vol.) as the developing solvent. The separation could be satisfactorily carried out on a semi-preparative scale without appreciable alterations in the R_F of the materials. Quantities of material up to 50 mg. have been successively fractionated. Paper chromatography on paper impregnated with silicic acid was carried out as described by Lea, Rhodes & Stoll (1955). Other paper-chromatographic methods have been described previously, together with the radioautographic techniques used (Hunter *et al.* 1959).

Column chromatography. Chromatography on silicic acid was carried out according to Hirsch & Ahrens (1958). After the fractions containing neutral lipids were removed, the phospholipid fraction was usually obtained by eluting the material remaining on the column with methanol.

Columns of diethylaminoethylcellulose (DEAE-cellulose) were prepared for ion-exchange partition chromatography in water saturated with butan-1-ol, and were washed with the same solvent. The lipid extracts were applied in butan-1-ol saturated with water, and fractions were collected by elution with the same solvent, followed by methanol, methanol–acetic acid (1:1, v/v) and, finally, aqueous acetic acid (90%, v/v).

Assays of radioactivity. Protein and other solid samples were prepared for radioactive assay as described previously, and end-window counting of the samples was carried out as before (Butler *et al.* 1958; Crathorn & Hunter, 1957).

Samples of column fractions were usually dried down on aluminium planchets and assayed at zero self-absorption by the same end-window apparatus; 1 μmc of a [^{14}C]amino acid gave a count of approx. 150/min. under these conditions. Samples eluted from paper chromatograms were assayed in the same way.

Materials. Generally-labelled L-amino acids (5–9 μc/μmole) were supplied by The Radiochemical Centre, Amersham, Bucks, and were used as supplied without any dilution with [^{12}C]amino acids. Silicic acid for chromatography was obtained from L. Light and Co. Ltd., Colnbrook, Bucks. DEAE-cellulose was supplied by Kodak Ltd., Kirkby, Liverpool.

RESULTS

Formation, extraction and fractionation of the labelled lipo-amino acid complexes

In the initial experiments, labelled amino acids were incubated with protoplasts for short periods, and after rapid cooling the protoplasts were sedimented and extracted with methanol or acetone. After the solvent was removed, the extracted material was subjected to paper chromatography followed by radioautography. The radioautograms showed in every case a prominent labelled spot running with a higher R_F than the amino acid added. These labelled spots corresponded to areas staining with Sudan black (Swahn, 1952) and are probably best termed lipo-amino acid complexes. The mobilities of the lipo-amino acid complexes derived from three of the amino acids most frequently used are given in Table 1. The absolute R_F values are liable to vary quite considerably in the very volatile solvent unless it is freshly made up.

Table 1. *Mobilities of some amino acids and the corresponding lipo-amino complexes*

The solvent system used was butan-2-one–acetic acid–water (3:1:1, by vol.). All measurements were made by upward flow.

Amino acid	R_F of amino acid	R_F of complex
Arginine	0·16, 0·25 (2 spots)	0·62
Phenylalanine	0·85	0·95
Threonine	0·60	0·85

Two studies illustrated in Fig. 1 show the time course of the labelling of lipid, protein and RNA fractions of protoplasts. It can be seen that the specific activity of the cellular lipid is relatively high at early times. The actual total radioactivity of the lipid very soon drops below that to be found in the protein, although, in one experiment where 100 mg. of protoplasts were incubated in 25 ml. of medium with $3\mu c$ of L-[^{14}C]phenylalanine for only 15 sec. before adding 200 ml. of ice-cold methanol, the lipo-amino acid complex acquired a total radioactivity of $19\mu mc$, whereas only $6\mu mc$ was found in the total trichloroacetic acid-precipitable material left after the removal of the lipo-amino acid.

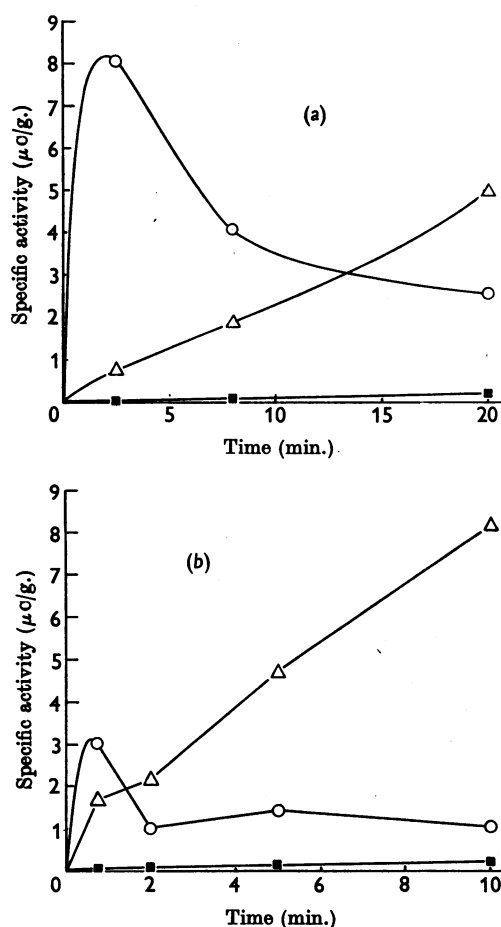Fig. 1. Uptake of amino acids into the lipid (O), protein (△) and RNA (■) fractions by protoplasts. (a) Protoplasts, (1·5 g.) incubated with $30\mu c$ of L-[^{14}C]arginine; (b), 1·4 g. of protoplasts incubated with $20\mu c$ of L-[^{14}C]threonine. Samples were taken at the indicated times, and other manipulations were as described in the text.

There was no doubt that the labelled amino acid was tightly bound in the lipo-amino acid complex. Elution of the labelled spots from the paper with methanol, equilibration of the eluted material with a large excess of the free unlabelled amino acid at room temperature either in aqueous or methanolic medium, and subsequent rechromatography again gave a complete separation of the lipo-amino acid complex from the free amino acid, without any loss of radioactivity or alteration in R_F. Subsequent work (see below) on column chromatography has incidentally confirmed this result many times over. The label was fixed as the unchanged amino acid residue, for the amino acid could be recovered in almost quantitative yield (in terms of ^{14}C) by incubating the lipo-amino complex with 0.1N-sodium hydroxide for 30 min. and then hydrolysing overnight with 6N-hydrochloric acid. The amino acid was then detectable by chromatography and radioautography in all three cases (alanine, phenylalanine and arginine) investigated. For some reason not fully understood, a direct hydrolysis with acid failed to give a quantitative recovery of the amino acid, but this may perhaps be related to an insolubility of the unaltered lipo-amino acid complex in aqueous media.

Column chromatography on silicic acid was then applied to determine in which fraction of the lipid the radioactivity resided. The main fractions of neutral lipids were eluted with mixtures of light petroleum and ether, and were essentially un-labelled. The 'monoglyceride' fraction eluted with pure ether also contained, with one exception, very little ^{14}C, and practically all the ^{14}C appeared in the 'phospholipid fraction' eluted with methanol. The one exception was when material originating from L-[^{14}C]phenylalanine was used. In this case, although the bulk of the neutral lipid eluted from the column according to the scheme of Hirsch & Ahrens (1958) was unlabelled, the final fraction obtained with 100% ether had a substantial radio-activity. L-[^{14}C]Phenylalanine also gave anomalous results, in that the lipo-amino acid complex obtained when a given quantity of this amino acid was used was always considerably more highly radioactive than that obtained from any of the other [^{14}C]amino acids used (alanine, aspartic acid, arginine, leucine, valine and threonine). The material eluted with ether in experiments with phenylalanine has not been examined more closely as yet, as it may well be an artifact (see below). The usual procedure in most experiments has been to remove the neutral-lipid fraction with ether and then elute the 'phospholipid fraction' with pure methanol. This fraction also contains free amino acids, but these can be removed by paper chromato-graphy as above either before or after the column chromatography.

Since methanol does not solubilize all the cellular phospholipid, the experiments were repeated with several extractions with methanol–chloroform (1:1, v/v), but no more labelled complex was extracted, even at room temperature, than when pure methanol was used. Further, nearly all the label could be extracted with acetone, and was in fact still soluble in dry acetone after the extract was freeze-dried, although the bulk of the phospholipid was insoluble under these conditions. This method of extraction has been used in most of the later experiments; in addition to a substantial separation from the bulk of the phospholipid, several of the free amino acids used (e.g. threonine) are almost completely insoluble in dry acetone. One exception was L-[14C]arginine; its lipo-amino acid complex was insoluble in acetone, and also could not be fractionated on silicic acid as it became firmly bound, presumably by salt-linkage with its guanidine groups.

In view of these results, it was not altogether surprising to find that the labelled material obtained in the methanol eluates from the silicic acid columns did not correspond in mobility to any of the common phospholipids when further fractionated by chromatography on paper impregnated with silicic acid. Thus the labelled material derived from L-[14C]phenylalanine ran with an R_F of 0·95 in the methanol–chloroform solvent of Lea *et al.* (1955). The material derived from L-[14C]threonine ran with a slightly lower R_F (0·9) but, in every case, a clear separation was effected from all the known classes of phospholipids with the exception of free phosphatidic acids. Although phosphatidic acids are substantially insoluble in acetone, it was neverthe-

less uncertain whether the labelled material eluted from the paper was contaminated with free phosphatidic acids. Alkaline hydrolysis of the eluted material gave glycerol and fatty acids (both identified chromatographically), and it is therefore possible that these substances could have originated from contaminating free phosphatidic acids rather than the complex itself.

This separation on paper impregnated with silicic acid was in any case unsuitable for processing other than small quantities of material, and a more satisfactory procedure has now been developed by using a combination of partition and ion-exchange chromatography on DEAE-cellulose. The methanol eluates from the silicic acid columns were dried down, dissolved in butanol saturated with water, and directly applied to the DEAE-cellulose columns. The DEAE-cellulose columns retain free amino acids under the conditions we have used and thus the material eluted consisted entirely of the lipo-amino acid complexes. The results of an experiment where the amino acid used was L-[14C]threonine are shown in Table 2A. Only the material in fraction 1 eluted with butanol contained much phosphorus, and careful re-running of fraction 1 showed that the label followed the phosphorus content and weight of eluted material. This first fraction was almost certainly phospholipid. The major part of the radioactivity was eluted in fraction 2 and contained no phosphorus. However, although the positions of the peaks of activity remained constant in different experiments, the proportion of label in fraction 1 was much greater in some experiments (e.g. that shown in Table 2B), and the amount of label in fraction 3 much less.

Table 2A. *Chromatography of the 'phospholipid fraction' on diethylaminoethylcellulose*

The chromatography was carried out as described in the Methods section on a column of DEAE-cellulose, 31 cm. × 1·7 cm. Whole cells (10·0 g.) were converted into protoplasts in five equal batches and incubated for 30 min. in the medium described (see Methods) before harvesting and extraction with acetone in the usual way. The extracts were then added to an extract similarly obtained from protoplasts derived from 0·8 g. of whole cells that had been further incubated for 5 min. with L-[14C]threonine (20 μc) before harvesting. The methanol eluate from the silicic acid column that was used in the preliminary removal of the neutral lipids contained 0·245 g. of material and a total radioactivity of approx. 350 μmc.

Fraction	Eluted with	Total radioactivity (μmc)	Weight (mg.)	P (%)
1	Butan-1-ol saturated with water	17·3	48·3	2·7
2	Methanol	293	149	0·11
3	Methanol–acetic acid (1:1, v/v)	59·8	26·5	0·66

Table 2B. *Chromatography of a second preparation of the lipo-threonine complex (721 mg.) as in Table 2A*

Fraction	Eluted with	Total radioactivity (μmc)	Weight (mg.)	P (%)
1	Butan-1-ol saturated with water	36	438	2·8
2	Methanol	40	160	0·0
3	Methanol–acetic acid (1:1, v/v)	30	61	0·64

Possibly fractions 2 and 3 represent degradation products of fraction 1. When material obtained from experiments with other labelled amino acids was used, two major fractions were again always obtained, but in some cases (e.g. that of leucine) two peaks of activity were obtained in the butanol fraction.

Origin and function of the lipo-amino acid complexes

The lipo-amino acid complexes extracted with acetone appear to be substantially free from artifacts. Thus they are not formed at all when the labelled amino acid is added only at the same time as the acetone. Further, if the incubation of the protoplasts with the labelled amino acid is carried out in the presence of enough chloramphenicol to inhibit protein synthesis, the labelling of the lipo-amino acid complexes is decreased to a low level. Phenylalanine is again exceptional, in that the decrease is only to 20% rather than 0–5% of the control figure, and it seems that the formation of that part of the lipo-phenylalanine complex that is eluted from silicic acid with ether is little affected

by chloramphenicol. However, even with this amino acid the decrease in labelling is very substantial. As shown in Table 3, chloramphenicol does not greatly affect the accumulation of free amino acids by the protoplasts, so that the lipo-amino acids do not presumably function in gross transport processes. Incidentally, crotoxin (phospholipase A) also inhibits the labelling of the lipo-threonine complex in parallel with its inhibition of protein synthesis.

The extent of labelling of the lipo-amino acid complexes can be altered in any one experiment by varying the time and the relative weights of protoplasts and added amino acid. As shown in Fig. 2, with phenylalanine, over 5% of the added amino acid can be rapidly transformed into lipo-amino
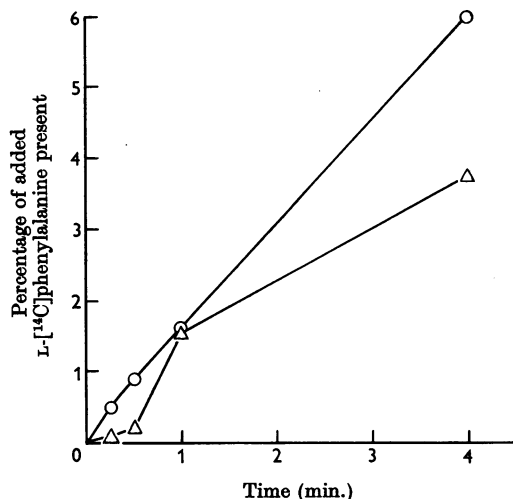
Table 3. *Effect of chloramphenicol on the accumulation of* L-[14C]*leucine by protoplasts of* Bacillus megaterium

In each experiment 60 mg. of protoplasts were incubated with 2μc of L-[14C]leucine for 15 min. at 30°, and, at the end of this period, half of the protoplasts was collected and freeze-dried for assay of total activity; the other half was precipitated with trichloroacetic acid, for estimation of the radioactivity of the protein, after lipids and nucleic acids had been removed by the usual procedures.

Chloramphenicol	Total radioactivity (μmc) present in		Total radioactivity in the 'free amino acid pool' (μmc)
	Protein	Whole protoplast	
Absent	1080	1660	580
Present (200 mg./ml.)	43	790	747

Fig. 2. Conversion of phenylalanine into the lipo-amino acid complex and protein. Protoplasts (400 mg.) were incubated with 12μc of L-[14C]phenylalanine, and cold methanol was added to samples at the indicated times. The other manipulations were as described in the text. O, Lipo-amino acid complex; △, protein.

Table 4. *Apparent passage of* [14C]*phenylalanine residues through the lipo-amino acids during protein synthesis by protoplasts*

In each experiment 0·47 g. of protoplasts was incubated with 3·7 μc of L-[14C]phenylalanine, and 50 mg. of L-[12C]phenylalanine was added at the indicated times. Other conditions and manipulations were as described in the text. The total radioactivity of the lipo-amino acids was determined by the elution and assay of material separated paper chromatographically; that of the protein was determined by removing lipid and nucleic acids from trichloroacetic acid precipitates in the usual way.

Time of incubation with L-[14C]phenylalanine before adding L-[12C]phenylalanine	Fraction	Total radioactivity (μmc) present after a further		Increase in radioactivity (μmc)
		5 min.	15 min.	
45 sec.	Lipo-amino acid	26·9	10·9	− 16·0
	Protein	42·0	52·0	+ 10·0
3 min.	Lipo-amino acid	34·0	13·3	− 20·7
	Protein	96·0	120·0	+ 24·0

acid complex in a few minutes, provided that the weight of added amino acid is large in relation to the weight of protoplasts. On the other hand, in an experiment where 1 g. of protoplasts was incubated with only 53 μg. of L-[^{14}C]phenylalanine (2·67 μc), nearly all the label was converted into protein in 5 min. and the label in the lipo-amino acid complex dropped to only 2 μmc, after an initial rise (at 1 min.) to at least 32 μmc. Thus it seemed that the label of the lipo-amino acid complex could be used for protein synthesis. An experiment (Table 4) where the labelled amino acid was followed shortly afterwards by a large excess of the corresponding [^{12}C]amino acid could also be interpreted most simply on the same basis. After enough time for the unlabelled amino acid to penetrate to the site of formation of the lipo-amino acid complex had elapsed, samples were taken and the total radioactivity in the lipo-amino acid complex and in the protein was measured. Further incubation then resulted in the gain in label of the protein being very close to the loss in label of the lipo-amino acid complex.

Finally, it has been shown that protoplasts can convert the isolated lipo-amino acid complexes into protein. Variable results were obtained in initial experiments, until it was found that conversions of the order of 20–60% could be obtained if the complexes were added to the protoplast medium in olive oil. In further experiments (Table 5), fraction 2 of the lipo-threonine complex from the DEAE-cellulose column was converted into protein by protoplasts in approx. 70% yield, and this conversion was not depressed when a large weight of unlabelled L-threonine was simultaneously added to the medium.

DISCUSSION

Lipo-amino acids have been isolated from a variety of cell types in recent years, and a few of the better-documented cases will be mentioned here. Čmelik (1955) has extracted complexes of phospholipids and amino acids from typhoid bacteria, and

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Table 5. *Incorporation of an isolated fraction of the lipo-threonine complex into protein by protoplasts*

Protoplasts (40 mg. in each treatment) were incubated at 30° for 30 min. Fraction 2 (see Table 2) of the lipo-threonine complex (8·3 μmc) was added with or without 10 mg. of L-[^{12}C]threonine in 0·4 ml. of olive oil in each experiment, and the incubation continued for a further 30 min. before it was terminated by the addition of trichloroacetic acid.

L-[^{12}C]Threonine	Total radioactivity in the isolated protein (μmc)	Conversion of lipo-threonine into protein (%)
Present	6·0	72
Absent	6·3	75

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similar complexes have been found in extracts of *Penicillium chrysogenum* (Gaby, Naughten & Logan, 1959). Westley, Wren & Mitchell (1957) found that most of the non-protein amino acids in young larvae of *Drosophila melanogaster* are to be found in a lipid-soluble fraction. Hendler (1959) has found lipo-amino acids in minces of hen-oviduct, whereas, more recently, complexes of amino acids and phospholipids have been isolated by Gaby & Silberman (1960) from rabbit-liver and by Wallach, Soderberg & Bricker (1960) from ascites-tumour cells. Wren (1960) has considered the question whether these complexes might arise as artifacts in the course of extraction procedures. His conclusions, based largely on experiments carried out with *D. melanogaster*, were that although some lipo-amino acids are artifacts, others do exist *in vivo*. Gaby & Silberman (1960) have also taken pains to show that the complexes they isolated from rabbit liver were formed metabolically. On the other hand, Haining, Fukui & Axelrod (1960) considered that the lipo-amino acid complexes they extracted from cellular fractions of rat liver were mainly artifacts. However, the latter group of workers were following the incorporation of labelled amino acids into a relatively inactive system which probably favoured artifact formation, and in any case they made little attempt to fractionate the gross lipid extract that they obtained. In the present work, the experiments with chloramphenicol in particular make it unlikely that the bulk of the lipo-amino acid material studied was an artifact.

Thus it seems fairly well established that some lipo-amino acids are normal cellular metabolites, and the next question that arises is that of their function in the cell. Gaby *et al.* (1959) thought that the complexes formed in *P. chrysogenum* occurred to such an extent and were so rapidly metabolized that they must play an important part in the metabolism of amino acids by the cell. Hendler (1959) went considerably further and suggested, largely as a result of kinetic studies of the passage of [^{14}C]amino acids through lipoidal fractions extracted from the hen-oviduct, that they might well function as intermediates in protein synthesis. In this connexion, Hendler (1960) has briefly reported that similar complexes have been found in ribosomes from *Escherichia coli*. This is in accord with the results obtained by Westley *et al.* (1957) who showed that the lipo-amino acid complexes found in larvae of *Drosophila* strikingly diminished in amount as protein was synthesized.

The results reported here again lead to the conclusion that the amino acid residues of lipo-amino acids can be used for protein synthesis. It is, of course, possible that they are not obligatory intermediates in protein synthesis but are storage forms of activated amino acids. But in that case it seems

unlikely that they should be formed in protoplasts capable of very rapid protein synthesis, and that the process of their formation should be sensitive to the action of chloramphenicol, which does not inhibit the formation of activated amino acids or their binding to RNA (Hunter *et al.* 1959). The rate of labelling of the RNA–amino acids complexes is roughly linear; similar findings have been reported by Hendler (1959). As he pointed out, this is not what would be expected of a precursor of protein, but it is difficult to draw any definite conclusion, as the material extracted is presumably a complex mixture, including, possibly, much RNA that is biologically inert.

Our main conclusions, therefore, are that lipo-amino acids are normal cellular metabolites of *B. megaterium* and that the amino acid residues so bound can readily be used for the synthesis of protein without prior conversion into free amino acids.

SUMMARY

1. When protoplasts of *Bacillus megaterium* are incubated with [^{14}C]amino acids in growth medium, some of the amino acid is bound tightly in the form of a lipo-amino acid complex or complexes, which can readily be extracted by acetone or methanol and separated from free amino acids chromatographically. The amino acids may be recovered unchanged from the complexes by hydrolysis.

2. When chromatography on silicic acid is carried out, the labelled lipo-amino acids can be eluted with methanol in the 'phospholipid fraction'. Very little radioactivity can normally be eluted with the neutral lipid fractions.

3. The labelled lipo-amino acid complexes can be further separated into phospholipid and phosphorus-free fractions by chromatography on di-ethylaminoethylcellulose.

4. The labelled lipo-amino acid complexes studied here are not formed when the incubations are carried out in the presence of chloramphenicol or crotoxin, or when the labelled amino acid is added at the time of solvent extraction.

5. Various experiments suggest that the radioactivity bound in both intrinsic and added lipo-amino acid complexes can be used directly for protein synthesis by protoplasts, apparently without prior hydrolysis to free amino acids.

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