smaller values of p_1 would require larger value of R or M . One can only try to estimate the value of b , the initial radius of the column of radicals, by analogy with the results of ion recombination in gases. From these we would infer that we should use a value of about 2×10^{-7} cm., the value is low because we are using a columnar theory, whereas the ions (and probably also the radicals) are formed in clusters along an electron track (see Kara-Michailova & Lea, 1940). Using this value for b and $Z = 4 \times 10^{-10}$ cm.³sec.⁻¹, we have to assume $p = 0.003$ for the non-chain reaction, or $p = 0.019$ for the chain reaction, both cases therefore giving a low affinity of the radicals for the solute.

SUMMARY

1. Experiments are described showing the deamination by X-rays of some amino-acids and their derivatives, polypeptides and related nitrogenous

compounds. The relationship between the ionic yield and radiation dose has been studied in detail for glycine. The ionic yield rises from 0.3 to 2.9 for very dilute to nearly saturated solutions. The ionic yield for dry glycine was 2.96.

2. An explanation of the high ionic yield with glycine is attempted in terms of the radical hypothesis assuming either a chain reaction or the formation of radicals by excitation in addition to charge neutralization. The continuous rise of the yield is interpreted as being caused by recombination of radicals competing with deamination even at the highest concentrations of glycine, due to a low affinity of glycine to radicals. The explanation offered, although consistent with the experiments described, is not wholly satisfactory in view of the radiation effects on thiourea.

3. The biological significance of the results is discussed.

REFERENCES

- Allsopp, C. B. (1944). *Trans. Faraday Soc.* **40**, 79.
 Allsopp, C. B. (1948). *Brit. J. Radiol.* **21**, 242.
 Auerbach, C. & Robson, J. M. (1947). *Proc. Roy. Soc. Edinb. B*, **62**, 284.
 Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, 2nd ed. London: Crosby, Lockwood and Son, Ltd.
 Dainton, F. S. (1948). *J. phys. colloid. Chem.* **52**, 490.
 Dale, W. M. (1942). *Biochem. J.* **36**, 80.
 Dale, W. M. (1943). *Brit. J. Radiol.* **16**, 171.
 Dale, W. M. (1947). *Brit. J. Radiol. Suppl.* No. 1, 46.
 Dale, W. M. & Davies, J. V. (1949). *Nature, Lond.*, **163**, 64.
 Dale, W. M., Davies, J. V. & Meredith, W. J. (1949). *Brit. J. Cancer*, **3**, 31.
 Dale, W. M., Gray, L. H. & Meredith, W. J. (1949). *Philos. Trans. A*, **242**, 33.
 Dale, W. M., Meredith, W. J. & Tweedie, M. C. K. (1943). *Nature, Lond.*, **151**, 280.
 Jaffé, G. (1913). *Ann. Phys., Lpz.*, **42**, 303.
 Kara-Michailova, E. & Lea, D. E. (1940). *Proc. Camb. phil. Soc.* **36**, 101.
 Lea, D. E. (1946). *Action of Radiations on Living Cells*. Cambridge: University Press.
 Lea, D. E. (1947). *Brit. J. Radiol. Suppl.* No. 1, p. 59.
 Lea, D. E., Smith, K. M., Holmes, B. & Markham, R. (1944). *Parasitology*, **36**, 110.
 Lobashov, M. E. (1937). *Genetics*, **19**, 200.
 Miller, N. (1948). *Nature, Lond.*, **162**, 448.
 Piaggio, H. T. H. (1933). *Differential Equations*. London: Bell.
 Putnam, F. W. & Neurath, H. (1946). *J. biol. Chem.* **166**, 603.
 Stein, G. & Weiss, J. (1948). *Nature, Lond.*, **162**, 184.
 Weiss, J. (1944). *Nature, Lond.*, **153**, 748.
 Weizmann, C. H., Bergmann, E. & Hirschberg, Y. (1936). *J. Amer. chem. Soc.* **58**, 1675.

An Investigation of the Intracellular Fluid of Calf Embryo Muscle

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Van Slyke (1913) estimated the amount of amino nitrogen extractable with boiling water from the skeletal muscle of the dog. Very few attempts have, however, been made to estimate or even to identify the particular amino-acids present in the intracellular fluid of any tissue. The work on muscle has been summarized by Ussing (1943), who mentions estimations of arginine, histidine, cystine, glutamic acid, tyrosine and leucine plus valine. Since then Christensen, Streicher & Elbinger (1948) have

estimated glycine and glutamine in guinea pig muscle.

The present investigation concerns the substances in calf embryo muscle with molecules small enough to pass through a dialysis membrane; these mixtures are also compared with similar ones obtained from adult ox muscle and serum. Embryo muscle was used because extracts of this material are employed to maintain the growth of tissue cultures; although the stimulating effect of embryo extracts may be due

only to the large molecules present (Fischer, Astrup, Ehrensvärd & Øhlenschläger, 1948) it seemed of interest to try to ascertain whether there is any substance of low molecular weight detectable in embryo muscle but absent from that of the adult. The method used, that of partition chromatography on paper, while not suitable for quantitative estimations (with certain exceptions to be mentioned below), is particularly useful for an extensive survey.

EXPERIMENTAL

Investigations of intracellular fluids are made difficult by the fact that interference with the cell must undoubtedly lead to changes in its contents. Also the method used for the separation of the smaller from the larger molecules may have considerable effect on the results finally obtained. In the present work the tissue was minced and subjected to dialysis in the cold as described by Astrup, Ehrensvärd, Fischer & Øhlenschläger (1947). This technique was chosen because it seemed less likely to cause chemical change than either denaturation of the proteins by boiling (Fuller, Neuberger & Webster, 1947) or extraction with trichloroacetic or picric acid (Christensen *et al.* 1948). It has the advantage over extraction with ethanol (Awapara, 1948) that there is no danger of the amino-acids being rendered insoluble or converted into esters. The chief disadvantage of this technique is that some enzymic breakdown of the large molecules is bound to take place during the dialysis. This is probably rather small in the case of the muscle proteins as it has been shown by Greenstein & Leuthardt (1946) that this tissue is low in proteolytic enzymes. However, as it would clearly be undesirable to carry on dialysis for too long a period, complete separation of the small molecules is impracticable.

When the solution obtained in this way was subjected to two-dimensional chromatography two spots which could not at first be recognized were obtained in that part of the chromatogram characteristic of acidic compounds. Therefore the original solution was fractionated on a column of Amberlite IR 4; both the substances responsible for the unknown spots were found to be retained and were thus separated from the neutral and basic amino-acids. Further investigation showed that the stronger of the two spots was due to aminoethylphosphate, a substance which had not previously been recognized as occurring in embryo muscle (Outhouse, 1937). The properties of the substance responsible for the other spot corresponded with those to be expected for a phosphoric ester of hydroxylysine. While the structure of this amino-acid has not yet been fully clarified (Van Slyke, Hiller, MacFadyen, Hastings & Klemperer, 1940) it is of interest that aminoethanol could be obtained from either of the

suggested structures by fission at the linkage between the γ - and δ -carbon atoms. For convenience the hydroxylysine-containing compound of the muscle extract will subsequently be referred to as hydroxylysinephosphate. As hydroxylysine has not previously been reported in combined form this substance has been further investigated. A preliminary report of its occurrence has already appeared (Gordon, 1948).

An attempt at a quantitative estimation of aminoethylphosphate and hydroxylysinephosphate has also been made by taking advantage of the ease with which these substances can be separated from other phosphorus-containing compounds and from one another on one-dimensional paper chromatograms. These analyses were performed on the fractions obtained from the Amberlite chromatogram, thus obviating the need to subtract the inorganic phosphorus which remained absorbed on the Amberlite until the final treatment with acid. Three fractions containing aminoethylphosphate and hydroxylysinephosphate were estimated separately so as to get information on the order of elution of these substances from the Amberlite. Each paper strip was cut up and eluted by the technique of Consden, Gordon & Martin (1947), guide chromatograms being used for the location of the spots. It would be simpler to treat the whole chromatogram with ninhydrin, but in the present work guides were used as a convenient means of keeping down the amounts of extraneous phosphorus in the eluates.

Preparation of material. As much as possible of the skeletal muscle was removed from a calf embryo weighing approximately 4 kg. After mincing, this weighed 622 g. An equal volume of water was added and the mixture was placed inside a cellophan tube and was allowed to dialyse with rocking at 5° against 2.4 l. of water for 20 hr. The solution outside the membrane was then removed (solution 1), replaced by a further 2.4 l. of water, and the dialysis repeated (solution 2). After solutions 1 and 2 had been concentrated *in vacuo* below 40°, estimations of N by micro-Kjeldahl, and of total and inorganic P by the method of Kuttner & Lichtenstein (1932) were made. The results are shown in Table 1. Solutions 1 and 2 were then combined and will be referred to as the embryo muscle dialysate.

Table 1. *Nitrogen and phosphorus obtained by dialysis of embryo mince*

	Nitrogen (mg. N)	Phosphorus (mg. P)	
		Inorganic	Organic
Solution 1	393	100	88.0
Solution 2	214	72.5	34.3
Total	607	172.5	122.3

Two-dimensional chromatography of embryo muscle dialysate. Most of the inorganic salts present were removed from part of the above solution by the electrodialytic method of Consden *et al.* (1947). An amount of the solution thus obtained

containing 0.52 mg. N was subjected to two-dimensional chromatography using phenol for 17 hr. followed by *s*-collidine for 42 hr. The experimental conditions were the same as those described by Consden, Gordon & Martin (1944), except that 0.003% (w/v) of 8-hydroxyquinoline was added to the phenol, and coal gas was omitted. Whatman no. 4 filter paper was always used. A solution of 0.3% (w/v) NH_3 in the aqueous phase was employed at the bottom of the chamber for all the chromatography in phenol described in the present paper, except where an atmosphere containing acetic acid was used. Previous trials, in which the order of the solvents was reversed, gave much inferior results, confirming the observations of Dent (1947) with mixtures of this kind. The resulting chromatogram is shown in Fig. 1.

As a check on the identification of certain spots some of the muscle dialysate was made 6N with HCl and refluxed for 21 hr. After removal of the HCl *in vacuo* a similar two-dimensional chromatogram was prepared.

Fig. 1. Two-dimensional chromatogram of calf embryo muscle dialysate. Developed for 17 hr. in phenol followed by 42 hr. in *s*-collidine. For explanation of the spots see Table 2.

Preparation and chromatography of adult ox muscle dialysate. A specimen of muscle from a fully grown animal was minced and extracted as described above for the embryo muscle. After removal of salt, a similar two-dimensional chromatogram was prepared from an amount of solution containing 0.94 mg. N; this rather large amount of material was used in order to show up any traces of hydroxylysine-phosphate which might have been present.

A spot which appeared in the position expected for glutathione was easily separable from all other spots on a one-dimensional chromatogram using phenol, and hence it could conveniently be investigated by the elution technique of Consden *et al.* (1947). The eluate from a chromatogram developed in this way was hydrolysed, brominated and reinvestigated on a second one-dimensional chromatogram using phenol.

Preparation and chromatography of adult ox serum dialysate. The blood of an adult animal was defibrinated by stirring and the erythrocytes were removed by centrifugation. The serum thus obtained was dialysed as described above. After removal of salt from the material which had passed through the membrane, a similar two-dimensional chromatogram was prepared using 0.53 mg. N. The spots observed on the various two-dimensional chromatograms are recorded in Table 2.

Table 2. *Strengths of spots observed on two-dimensional chromatograms of calf embryo muscle dialysate (0.52 mg. N), adult ox muscle dialysate (0.94 mg. N) and adult ox serum dialysate (0.53 mg. N)*

(As the various amino-acids give different amounts of colour with ninhydrin, uncorrected comparisons are only valid for the same amino-acid. For the minimum amounts of each amino-acid visible by means of ninhydrin on paper see Pratt & Auclair (1948). The numbers in brackets after each amino-acid spot observed on the embryo muscle chromatogram refer to the spot numbers in Fig. 1.)

Strength of spot	Embryo muscle	Adult muscle	Adult serum
Very strong	Alanine (9) Glutamic acid (4) Glycine (6)	Alanine Glutamic acid Taurine	Alanine Glycine Leucine + iso-leucine
Strong	Aminoethylphosphate (5) Glutamine (8) Taurine (11)	Carnosine Glutamine Glutathione Glycine Leucine + iso-leucine	Glutamic acid Lysine Valine
Medium	β -Alanine (12) Aspartic acid (3) Carnosine (19) Leucine + iso-leucine (22)	Aspartic acid Lysine Serine Valine	Aspartic acid Arginine Glutamine Proline Serine
Weak	Arginine (18) Lysine (17) Phenylalanine (23) Hydroxylysine-phosphate (1) Proline (20) Serine (7) Tyrosine (24) Valine (21)	β -Alanine Arginine Methionine sulphoxide Aminoethyl-phosphate Proline Tyrosine	γ -Amino-butyric acid Methionine sulphoxide Phenylalanine Taurine Threonine Tryptophan Tyrosine
Very weak	α -Aminobutyric acid (14) γ -Aminobutyric acid (15) Glutathione (2) Hydroxyproline (13) Methionine sulphoxide (16) Threonine (10)	Phenylalanine	α -Aminobutyric acid Histidine

Chromatography of embryo muscle dialysate on Amberlite IR4. The method employed was the same as that of Consden, Gordon & Martin (1948) except for certain simplifications. Thus the Amberlite was used in the form obtained from the manufacturers without preliminary grinding and sieving. After alternate treatments with dilute HCl and NH_3 the

excess acid was removed from the chromatogram by elution with distilled water. Using a column 32×3.7 cm. this required about 5 days, the column being ready for use when the eluate became 0.0013 *N* (ascertained by titration).

Embryo muscle dialysate (50 ml.) containing 404 mg. N was applied to the chromatogram at a rate of not more than 150 ml./hr. After it had run in, it was followed by water at the same rate. Fractions were collected as shown in Table 3, the last fraction being eluted with 1 l. of *N*-HCl. Each fraction was concentrated *in vacuo* and estimations made of N and of inorganic and total P. One-dimensional chromatograms using phenol were also developed from samples of each fraction, salt having been removed electro-dialytically from fraction 1. The results shown in Table 3 indicate that aminoethylphosphate and hydroxylysinephosphate, under these conditions, are somewhat absorbed on Amberlite, although not so much as are aspartic and glutamic acids. In a preliminary experiment it was found that a similar chromatogram will separate hypoxanthine from most of the neutral amino-acids. The large white precipitate obtained on concentrating fraction 2 of the present experiment probably consisted mainly of this substance.

Further investigation of fractions from the Amberlite chromatogram

Phosphorus estimations on eluates from paper chromatograms. 20 μ l. of each of fractions 2, 3 and 4 containing respectively 48.5, 28.4 and 20.8 μ g. P were developed on one-dimensional paper chromatograms using phenol for 17 hr. After drying, the chromatograms were cut up and eluted as described by Consden *et al.* (1947) except that about 1 ml. of water was

allowed to run through each cut directly into a tube suitable for incineration. The P estimations were done as described by Kuttner & Lichtenstein (1932), except that half quantities of each reagent were used. The results are shown in Table 4. In order to indicate the limitations of the method the small amounts of P which originate from the paper have not been subtracted from the figures in columns 3, 6 and 9 of Table 4. Control experiments showed that an average of 0.05 μ g. P could be eluted from a 1×10 cm. piece of Whatman no. 4 filter paper, down which nothing but the solvent has passed. If this correction is made, recoveries of 85, 101 and 93% are obtained by summing the P eluted from the whole of each chromatogram. In a control experiment on a sample of aminoethylphosphate (kindly supplied by Dr C. E. Dent) applied to a similar chromatogram, a recovery of 97% was obtained, using the same corrections.

Qualitative investigations of aminoethylphosphate and hydroxylysinephosphate

The materials used were eluted from one-dimensional chromatograms similar to those used for the quantitative investigation described above. In order to obtain a larger amount, 0.3 ml. of fraction 3 was applied to a sheet of paper 48 cm. wide. Only the cuts which were expected to contain aminoethylphosphate or hydroxylysinephosphate were eluted. The solution of the latter obtained in this way was further purified by a similar chromatographic procedure in which *n*-butanol containing 20% of acetic acid was used instead of phenol. In a second experiment *s*-collidine was substituted for *n*-butanol-20% acetic acid, without noticeably affecting the product.

Table 3. *Fractions obtained from embryo muscle dialysate by chromatography on Amberlite IR 4*

	Fraction					Total
	1	2	3	4	5	
Volume of eluate (ml.):						
Water	500	240	1640	360	—	2740
<i>N</i> -HCl	—	—	—	—	1000	1000
N as % of total N	73.8	6.9	2.8	1.0	8.7	93.2
Inorganic P as % of total inorganic P	—	—	—	—	91.4	91.4
Organic P as % of total organic P	7.0	1.4	11.4	1.4	72.7	93.9
Substances identified by chromatography on paper:						
Hydroxylysinephosphate	—	V. weak	Medium	Weak	—	—
Aspartic acid	—	—	—	—	Weak	—
Glutamic acid	V. weak	V. weak	Strong	V. strong	V. strong	—
Aminoethylphosphate	—	V. weak	Medium	Weak	—	—
Neutral and basic amino-acids	V. strong	V. weak	V. weak	—	—	—

Table 4. *Amounts of phosphorus eluted from cuts from paper chromatograms*

Cut no.	Fraction 2			Fraction 3			Fraction 4		
	Breadth (cm.)	P (μ g.)	P as % of total organic P	Breadth (cm.)	P (μ g.)	P as % of total organic P	Breadth (cm.)	P (μ g.)	P as % of total organic P
1	4.1	1.8	0.1	3.7	1.9	0.7	3.8	1.8	0.1
2	3.6	1.8	0.1	2.8	1.0	0.3	2.6	0.7	0.0
3	4.4	13.1	0.4	4.9	6.0	2.3	4.6	4.4	0.3
4	4.5	0.8	0.0	3.1	0.5	0.1	2.9	0.3	0.0
5	8.8	12.9	0.4	5.8	13.0	5.1	7.5	10.7	0.7
6	8.0	0.0	0.0	8.0	0.4	0.0	8.2	0.6	0.0
7	9.0	0.4	0.0	8.3	0.6	0.1	8.0	0.3	0.0
8	9.0	13.4	0.4	8.3	7.5	2.8	8.0	2.8	0.2
Total	51.4	44.2	1.4	44.9	30.9	11.4	45.6	21.6	1.3

Enzymic hydrolyses. The enzyme used was the phosphatase of dog faeces, purified according to Armstrong (1935), stages A and B, and subsequently dialysed. It was allowed to act for 3 hr. at 40° on each of the above eluates (approximately 10 μ g. of enzyme/ μ g. P). The pH of each solution was adjusted to 8.8 with NH_3 . After drying *in vacuo* the products were investigated on one-dimensional chromatograms using phenol, phenol + 0.003% 8-hydroxyquinoline in an acidic atmosphere (5% acetic acid at the bottom of the chamber) and *s*-collidine as solvents. The R_F values of the spots due to the original substances and those due to their hydrolysis products are shown in Table 5.

Table 5. R_F values of certain phosphorus-containing substances of calf embryo muscle

Solvent Addition ...	Phenol 0.003% 8-hydroxyquinoline + 0.3% NH_3	Phenol 0.003% 8-hydroxyquinoline + 5% acetic acid	<i>s</i> -Collidine
Hydroxylysine-phosphate	0.12	0.17	0.0
Hydroxylysine	0.66	0.22	0.14
Aminoethyl-phosphate	0.31	0.39	0.05
Aminoethanol	Front	0.67	0.22

The nature of the amino-acid set free from hydroxylysine-phosphate was further checked by admixture with a specimen of hydroxylysine kindly made available by Dr J. G. Heathcote. No separation could be obtained using any of the above solvents. The identity of the aminoethylphosphate was similarly checked both before and after hydrolysis with the enzyme, against a specimen kindly supplied by Dr C. E. Dent. Again no separation was obtained. As can be seen from Table 5 the difference between the R_F values of hydroxylysine and hydroxylysinephosphate in phenol-5% acetic acid is too small for complete separation to be easily possible. The results of such chromatography of samples of hydroxylysinephosphate after treatment with the phosphatase were often difficult to interpret since more than one spot could usually be observed which had moved more slowly than hydroxylysine and was incompletely separated from it. However, when a sample of hydroxylysinephosphate, treated with the phosphatase under the conditions described above, was developed on a two-dimensional chromatogram using phenol-5% acetic acid for 17 hr. followed by phenol-0.03% NH_3 for 17 hr., only one spot was observed. This had the expected R_F values for hydroxylysine.

Acid hydrolysis. Small amounts of the aminoethylphosphate and hydroxylysinephosphate were hydrolysed at 100° for 24 hr. in 6N-HCl as described by Consden *et al.* (1947). After removal of HCl, chromatography of the hydrolysate of aminoethylphosphate showed only spots corresponding to unchanged material and aminoethanol. From hydroxylysinephosphate were formed only spots corresponding to unchanged material and hydroxylysine and in phenol-acetic acid a faint spot (R_F 0.07) of unknown origin.

RESULTS AND DISCUSSION

Although the full characterization of the substance referred to in the present paper as hydroxylysinephosphate must await its isolation, perhaps from

a tissue in which it occurs in higher concentration, its presence in embryo and its absence from adult muscle remains the most interesting finding of the present work. How little hydroxylysinephosphate can be present in the adult muscle is indicated by the failure to obtain a spot due to this substance even though considerably more than the usual amount of the dialysate was used for this chromatogram. It is also of interest that while a spot due to aminoethylphosphate was formed from both the muscle extracts, that from the embryo was much the strongest. Neither of these substances was detectable in the serum of an adult animal. Rather strong spots due to aminoethylphosphate, but none due to hydroxylysinephosphate, were observed on chromatograms of similar dialysates made from Rous sarcoma (Mill Hill) and from Ehrlich mouse sarcoma. (The dialysis and chromatography of these two sarcomas was done by Ing. R. Stjernholm to whom thanks are due.)

The glutathione spot formed from the embryo muscle was very weak in contrast to that formed from the adult muscle. When the latter was separated and hydrolysed as described above, the hydrolysate on further chromatography yielded only spots due to glutamic acid, glycine and cysteic acid (from cystine). Thus the occurrence of serinephosphate, which has almost identical R_F values with glutathione in both solvents, was excluded. Not surprisingly, carnosine was absent from serum, but present in considerable amounts in both the tissue dialysates. Strong spots due to taurine were formed from both the tissue dialysates, while a weak spot appeared on the serum chromatogram. A spot due to this substance has been observed by Dent (1948) on chromatograms of human plasma.

Turning now to the α -amino-acids, it is noticeable that the concentration of leucine and/or isoleucine, valine and lysine in the embryo muscle dialysate is relatively low compared with the concentration of these amino-acids in serum. An intermediate amount seemed to be present in the adult muscle dialysate. On the other hand, glutamic acid and glutamine are rather stronger in the tissue dialysates than in the serum.

Very weak spots were detected on the chromatograms of embryo muscle extract and adult serum in the positions to be expected for α - and γ -aminobutyric acids. Also a weak spot, probably due to methionine sulfoxide, was observed on all three two-dimensional chromatograms. Dent (1948) has observed similar spots on chromatograms of various natural mixtures, and has checked their identity by the addition of known samples of each substance. However, until these substances have been isolated and fully characterized their recognition must remain somewhat tentative. In the present work such checks were not made. Confirmation was

obtained of the stability of the various substances towards acid by chromatographic examination of a sample of the embryo dialysate which had been refluxed for 17 hr. with 6N-HCl. Only the spots due to glutathione, glutamine and carnosine had disappeared.

Needham (1931) has summarized many investigations of the concentration of amino N in the blood of human and rabbit embryos. The amounts present were always rather greater than in the corresponding maternal blood. Although this work does not seem to have been extended to the tissue fluids of the embryo it is likely that these also must contain higher concentrations of amino-acids than do the maternal tissues. Even if such differences are shown to be the case it seems likely that they will be much smaller than the corresponding differences of concentration of hydroxylysinephosphate and aminoethylphosphate noticed in the present investigation between embryo and adult ox muscle. The only quantitative estimations of these substances so far attempted have been carried out on the fractions obtained from the embryo muscle extract after chromatography on Amberlite IR 4. If the amounts of P believed to be due to hydroxylysinephosphate in the various fractions are summed, it appears that this compound represents 3 % of the organic or 1 % of the total P of the embryo muscle dialysate. Similarly, aminoethylphosphate represents 6 % of the organic or 2 % of the total P. If unknown P-containing substances have moved at similar rates on the chromatograms used, these figures will of course be too high. That any correction would be small, however, is suggested by an experiment in which hydroxylysinephosphate, after elution from a phenol chromatogram, was rechromatographed using *n*-butanol-20 % acetic acid; on elution of appropriate sections of the second chromatogram no separation of P from the hydroxylysinephosphate was found.

In order to calculate the total quantities of these substances present in the muscle of the original embryo, account must be taken of the fact that the dialysis was not taken to completion. An indication of the proportion of the low-molecular-weight substances actually obtained can be gained from Table I which gives the amounts of N and P passing through the membrane after the successive periods of dialysis. Thus, if it is assumed that the same proportion of the N would have passed through the membrane if the

solution had been subjected to further successive dialyses, it appears that some 70 % of the non-protein N was removed from the mince. On a similar assumption 67 % of the available P was obtained. The considerably higher proportion of inorganic P obtained during the second period of dialysis is an indication of the rapid breakdown of organic phosphates which was taking place.

One of the objects of the present work was to test the effect on tissue cultures of any substance found to occur only in embryo muscle. This has not yet been attempted with the fractions containing hydroxylysinephosphate, but as aminoethylphosphate seemed to be so much more concentrated in the embryo muscle dialysate than in the adult, it was subjected to test. The system used was the usual one of Fischer & Astrup (1942) employing chick heart fibroblasts grown in a Carrel flask. Using the synthetic aminoethylphosphate + cystine, glycine and glutamine, excellent growth was obtained. Further experiments would be necessary to decide whether aminoethylphosphate is more than simply a good source of P for tissue cultures.

SUMMARY

1. The amino-acids and peptides of the intracellular fluids of calf embryo muscle have been examined by two-dimensional chromatography on paper and compared with similar mixtures from muscle and serum of the adult animal.

2. After removal of basic and neutral substances by chromatography on Amberlite IR 4 the acidic components were fractionated by one-dimensional paper chromatography. Sections corresponding to the spots observed on treatment with ninhydrin were eluted and the eluates were analysed for phosphorus and further examined.

3. Hydrolysis and repeated chromatography of these eluates indicated that aminoethylphosphate and hydroxylysine in a form combined with phosphorus are present. The former substance was found to represent 2 % and the latter 1 % of the dialysable phosphorus of the minced muscle.

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REFERENCES

- Armstrong, A. R. (1935). *Biochem. J.* **29**, 2020.
Astrup, T., Ehrensvärd, G., Fischer, A. & Øhlenschläger, V. (1947). *Acta physiol. Scand.* **14**, 197.
Awapara, J. (1948). *Arch. Biochem.* **19**, 172.
Christensen, H. N., Streicher, J. A. & Elbinger, R. L. (1948). *J. biol. Chem.* **172**, 515.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1947). *Biochem. J.* **41**, 590.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1948). *Biochem. J.* **42**, 443.

- Dent, C. E. (1947). *Biochem. J.* **41**, 240.
 Dent, C. E. (1948). *Biochem. J.* **43**, 169.
 Fischer, A. & Astrup, T. (1942). *Pflüg. Arch. ges. Physiol.* **245**, 41.
 Fischer, A., Astrup, T., Ehrensvärd, G. & Øhlenschläger, V. (1948). *Proc. Soc. exp. Biol., N.Y.*, **87**, 40.
 Fuller, A. T., Neuberger, A. & Webster, T. A. (1947). *Biochem. J.* **41**, 11.
 Gordon, A. H. (1948). *Nature, Lond.*, **162**, 778.
 Greenstein, J. P. & Leuthardt, F. M. (1946). *J. nat. Cancer Inst.* **6**, 203.
 Kuttner, T. & Lichtenstein, L. (1932). *J. biol. Chem.* **95**, 661.
 Needham, J. (1931). *Chemical Embryology*, 1st ed. Cambridge University Press.
 Outhouse, E. L. (1937). *Biochem. J.* **31**, 1459.
 Pratt, J. J. & Auclair, J. L. (1948). *Science*, **108**, 213.
 Ussing, H. H. (1943). *Acta physiol. Scand.* **6**, 222.
 Van Slyke, D. D. (1913). *J. biol. Chem.* **16**, 187.
 Van Slyke, D. D., Hiller, A., MacFadyen, D. A., Hastings, A. B. & Klemperer, F. W. (1940). *J. biol. Chem.* **133**, 287.

The Effect of Dietary Oleic and Palmitic Acids on the Composition and Turnover Rates of Liver Phospholipins

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Ozaki (1927) found that rats fed on diets containing 20% oleic acid gained weight, whereas rats on diets containing similar amounts of palmitic or stearic acids lost weight. These weight changes indicate differences in the metabolism of oleic acid as compared with that of the saturated fatty acids. Channon & Wilkinson (1936) have shown that on low-protein, high-fat diets, lipid accumulation in the liver of the rat varies directly with the proportion of saturated fatty acids in the dietary fat; their fatty liver-producing diets were deficient in choline and accumulation of fat was prevented by choline. It was suggested that choline was involved in the desaturation of fatty acids. The differences in accumulation of fat may have been due to different phospholipin turnover rates determined by the varying supply of saturated fatty acids.

In the experiments reported in this paper low-protein diets containing pure palmitic and oleic acids were fed to rats in such a way as to eliminate effects due to different food intakes or absorption coefficients of the fatty acids. An attempt was made to correlate the different lipid accumulations with differences in liver phospholipin turnover rates.

METHODS

Male albino rats of the Wistar strain were used. All animals were bred by sister-brother matings from a single pair. The starting weight chosen was 150 g. as younger animals showed excessive weight loss, and some died on the palmitic acid diet described in Table 1. The complication of an additional requirement for lipotropic factors for growth was also more likely to be avoided at this weight.

In a preliminary experiment to determine the percentage absorption of pure oleic and palmitic acids when fed at a level of 27%, it was found that 70% of the ingested palmitic

acid, and 90% of the oleic acid were absorbed. The proportions of fatty acid and cellulose powder in the two diets were therefore adjusted so that for both groups of animals the amounts of fat absorbed and the values of the diets as sources of energy were the same.

Table 1. *Composition of fatty acid diets*

	Oleic acid diet	Palmitic acid diet
Casein (Labco) (%)*	8	8
Fatty acid (%)	21	27
Glycerol (%)	3	3
Sucrose (%)	50	50
Yeast (%)	5	5
Salts (%)†	5	5
Cellulose powder (%)‡	8	2

* When choline supplements were given the casein was sprayed with a solution of choline chloride to give 30 mg. choline/10 g. diet.

† NaCl, 22 g.; CaHPO₄·H₂O, 130 g.; potassium citrate, 125 g.; MgSO₄·7H₂O, 30 g.; ferric citrate, 5 g.; 0.7 g. of trace mixture (KI, 12 g.; NaF, 10 g.; MnSO₄, 2 g. Cu₂I₂, 1 g.; KAl(SO₄)₂, 1 g.; ZnSO₄·7H₂O, 1 g. de Loureiro, 1931).

‡ Grade A, 100 mesh 'solka flocc'; a highly purified cellulose powder obtainable from Johnsen, Jorgensen and Wettre Ltd., London.

Animals were starved for 24 hr. and then placed on the diets for 12 days. The palmitic acid diet was not eaten so readily as the oleic acid diet and therefore approximate pair feeding between litter mates was adopted. Controls were fed *ad lib.* on a stock diet consisting of skimmed milk powder, 20%; ground yellow maize, 18%; rolled oats, 15%; barley meal, 15%; biscuit meal, 15%; wheat germ, 5%; palm kernel oil, 5%; dried yeast, 5% and bran, 2%. Each animal received 10 µc. of ³²P as Na₂H³²PO₄ in distilled water by intraperitoneal injection, and was killed by decapitation 4 hr. later. In order to reduce the effect of recently ingested fat on the specific activities of liver phospholipins, food and water were removed from the animals 4 hr. before the injection of ³²P. (Specific activity (SA) = % injected ³²P/mg. phosphorus.)