Table 1. VARIATION IN PERCENTAGE COMPOSITION OF FREE FATTY ACIDS DURING EMBRYOGENESIS OF *P. americana*

Fatty acid carbon No.	Days of development 0	7	14	21	28	35	38	40	42	Nymph
C–14:0	1·6	0·7	0·3	0·5	1·0	1·0	1·7	1·1	1·7	2·0
C–16:0	26·8	27·0	26·5	26·6	25·5	24·5	22·0	21·0	24·3	26·1
C–16:1	2·3	1·6	1·0	2·1	2·2	1·5	1·9	1·9	2·8	1·2
C–17:0	0·3	0·2	0·3	0·1	0·1	0·2	0·3	0·2	0·4	0·4
C–18:0	2·1	3·3	3·4	3·7	3·6	4·6	6·5	6·5	6·4	7·1
C–18:1	43·4	45·0	45·0	47·8	46·5	42·9	42·5	45·0	43·0	46·0
C–18:2	19·2	21·0	24·0	19·0	20·5	19·6	21·0	17·2	17·0	15·0
C–18:3	1·3	0·4	0·4	0·3	0·2	0·5	0·5	0·5	0·2	0·1

fatty acids with six to twenty carbon atoms in the chain were identified, although Table 1 includes only those acids which occurred in measurable quantities. Palmitic (C16), oleic (C18 : 1), and linoleic (C18 : 2) predominate in the free fatty acids of the cockroach embryo (in vertebrates they consist mainly of palmitic, stearic and oleic acids[10]). There is no apparent preferential use of any individual acid, though the pattern of linoleic (C18 : 2) and linolenic (C18 : 3) acids is compatible with Fraenkel and Blewett's assertion that one or both is essential for eclosion[11]. It is concluded from Table 1 that the various free fatty acids are catabolized in proportion to their quantitative occurrence as reported for the total fatty acids from the embryo[9], and also for free fatty acids in vertebrate tissues[12]. Thus, the quantitative and qualitative patterns of the free fatty acids provide an indication of the rate and extent of glyceride catabolism during embryogenesis.

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Polyamide Layer Chromatography of some Synthetic Food Colours

RECENT developments in the preparation of polyamide layers in our laboratory have facilitated the application of polyamide layer chromatography to many kinds of compounds besides phenolic compounds; for example, DNP amino-acids[1], oestrogens[2], chloramphenicol derivatives[3], sulphonamides[4] and indole derivatives[5] have been successfully separated. Here I shall describe its application to the analysis of certain synthetic food colours. All of them have functional groups which form hydrogen bonds with polyamide.

Many analyses of food colours have been made with paper and thin layer chromatography[6], and thin film electrophoresis has been used for coal tar food colours by Criddle *et al.*[7]. None of these techniques, however, has proved entirely satisfactory.

R_F values obtained with five solvent systems are given in Table 1 and a typical chromatogram is shown in Fig. 1. It can be seen that a sharp resolution of food colours is obtained with polyamide layer chromatography. I found that the addition of sodium *p*-toluenesulphonate is essential to give small and sharp spots. I have observed that the presence of as little as 2 per cent of the salt in the solvent mixture is sufficient, and that an increase of the percentage of the salt present effects the increase of R_F values observed. This throws open the possibility that compounds with a sulphonic acid group may be efficiently resolved by polyamide layer chromatography with the solvent mixture containing sodium *p*-toluenesulphonate because all the food colours which I have tested have a sulphonic acid group in the molecule except eosin. Eosin shows a circular spot without addition of sodium *p*-toluenesulphonate.

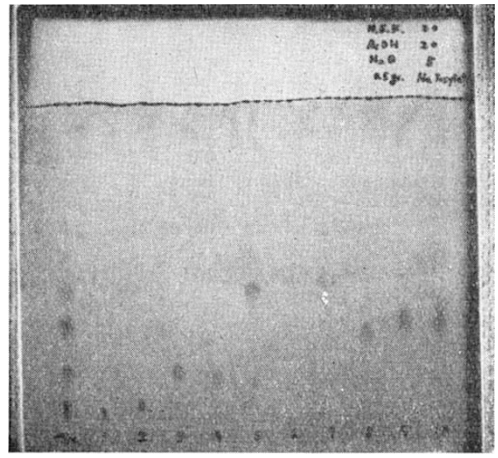

Fig. 1. Solvent: 2-butanone/glacial acetic acid/water/sodium *p*-tosylate v/v (40 : 40 : 10 : 1 g) layer, according to Wang (ref. 8) (Toyo Rayon Co., Amilan *CM* 1011 (26°)). Loading, 0·5 μg. Photographed by transmitted artificial light. *Key:* 1, New Coccine; 2, amaranth; 3, Ponceau *SX*; 4, tartrazine; 5, orange I; 6, eosin (sodium-salt); 7, naphthol yellow *S*; 8, sunset yellow FCF; 9, Ponceau 2*R*; 10, Ponceau 3*R*.

Table 1

No.	Solvents I	II	III	IV	V
(1) New Coccine	0·12	0·03	0·26	0·13	0·12
(2) Amaranth	0·14	0·06	0·43	0·22	0·14
(3) Ponceau *SX*	0·25	0·16	0·48	0·35	0·16
(4) Tartrazine	0·25	0·13	0·55	0·36	0·26
(5) Orange I	0·40	0·41	0·59	0·49	0·42
(6) Eosin (Na-salt)	0·53	0·64	0·67	0·61	0·54
(7) Naphthol yellow *S*	0·36	0·44	0·70	0·58	0·35
(8) Sunset yellow FCF	0·46	0·28	0·68	0·49	0·43
(9) Ponceau 2*R*	0·48	0·30	0·70	0·52	0·45
(10) Ponceau 3*R*	0·48	0·31	0·70	0·53	0·47

Solvents I, 2-butanone/glacial acetic acid/water/sodium tosylate v/v (20:40:10:1 g); II, 2-butanone/glacial acetic acid/water/sodium tosylate v/v (40:40:10:1 g); III, acetone/glacial acetic acid/water/sodium tosylate v/v (40:40:10: 1 g); IV, dioxane/glacial acetic acid/water/sodium tosylate v/v (40:40:10:1 g); V, *t*-butanol/glacial acetic acid/water/sodium tosylate v/v (20:10:5:1 g).

I found 10 cm development was sufficient because the spots were very small and sharp. The time required to ascend 10 cm from spot origin is 2·5–3 h except with the system containing *n*-butanol or *t*-butanol which required 6 h. The colours are very faint because of the small sample size (about 0·5 μg) and therefore they do not give good contrast on photographic paper; however, it is easy to locate the spots by transmitted daylight. Ponceau 2*R* and Ponceau 3*R* cannot be separated by these solvent systems probably because of their similarity in structures.

Polyamide layer is very durable and easy to handle. This layer with its sorption character is more suitable for analysing food colours than other adsorbents.

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