agar. The mixture is then poured into ordinary test tubes in 8–10 ml amounts. The tubes are slanted, left to harden at room temperature, then stoppered with rubber stoppers and sealed with paraffin. The pH of the medium is 7.6–7.8. Either PABA alone or PABA together with POB in various proportions was included in this medium by dissolving in Tyrode-serum mixture before filtration through a Seitz filter, previously washed thoroughly with distilled water. The agar powder employed was washed successively with 50% and 95% ethanol.

Agar-slant culture rickettsiae and virus were used as inocula. Tissue fragments (about 15 mg) from one culture tube showing maximum growth were collected with a spatula, mixed in a short test tube (15 × 80 mm) with about one g of tissue of 9–10 day-old chick embryo from which eyes and legs had been removed. The tissue was then minced well with scissors and deposited with a spatula upon the agar media, each culture tube receiving 60–70 mg of the tissue. Infective material obtained from one culture tube was usually used to inoculate fresh chick embryonic tissue for 12 new cultures, consisting of both control and test media.

The cultures were incubated at 35° C for 7–15 days, and growth was examined microscopically in Macchiavello-stained smears of cultured tissue.

TABLE 1

REVERSAL BY POB OF THE RICKETTSIOSTATIC ACTION OF PABA IN AGAR-SLANT TISSUE CULTURE

Medium		Growth after 8–15 days				
PABA (mg/100 ml)	POB (mg/100 ml)	*Rickettsia typhi*	*Rickettsia akari*	*Rickettsia rickettsii*	*Coxiella burnetii*	Psittacosis virus
0	0	+++	+++	+++	+++	+++
50	0	–	–	–	+++	+++
50	0.05	–	–	–	+++	+++
50	0.5	++	++(+++)	++(+++)	+++	+++
50	5	+++	+++	+++	+++	+++
100	0	–	–	–	+++	+++
100	0.1	–	–	–	+++	+++
100	1.0	++(+++)	++(+++)	++(+++)	+++	+++
100	10	+++	+++	+++	+++	+++
200	0	–	–	–	++·+++	+++

Degree of growth: –, none; + minimal; ++, moderate; +++, maximal.

In previous studies (*3*) it was shown that the growth of typhus rickettsiae is inhibited by PABA in deep columns of Maitland medium. The results presented in Table 1 demonstrate that the growth of three species of rickettsiae (*R. typhi, R. rickettsii,* and *R. akari*) is inhibited by PABA (0.5–1 mg/ml) in agar-slant tissue culture and that this inhibition can be reversed by POB in 1/100 the concentration of PABA. It may be noted that inhibition of *E. coli* by PABA is also reversed by POB in 1/100 the concentration of PABA (*5*). *C. burnetii,* which was reported to be least affected by PABA in the chick embryo (*9*), also is not readily susceptible to the action of PABA (1–2 mg/ml) on agar tissue culture. On a morphological, tinctorial, and cultural basis, the organism is a typical rickettsia. It requires living tissue to propagate, it stains similar to and looks like other rickettsiae. It distinctly differs from three other rickettsial organisms, however, in its susceptibility to the growth-inhibitory action of PABA, as well as in certain other biological characteristics (*10*).

Since the preservation of cell morphology of cultured tissue is, microscopically, quite satisfactory, and since both the virus of psittacosis and *C. burnettii* grow readily on agar media containing PABA (1-2 mg/ml), it is clear that the inhibitory action of PABA on the growth of the three species of rickettsiae is selective.

The agar-slant tissue culture technique has advantages and affords a procedure for studying the growth requirements of rickettsiae and viruses and for testing materials which may influence their growth.

Details of these and further studies will be published elsewhere.

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On the Presence of the Triphosphothiamine (TPT) in the Liver

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The presence in the liver of thiamine and of its diphosphoric ester (DPT) is too well known to require mention; we have obtained evidence for the existence of a triphosphoric ester of thiamine (TPT) in the liver of the albino rat by a chromatographic procedure. This is an adaptation of the methods suggested by Spadoni and Tecce (*1*) and by Viscontini and Karrer (*2*) for the separation of thiamine and of its phosphoric esters in pure solutions.

About 3–4 g of liver are homogenized in 3 vol of 0.2 *N* HCl with the Potter-Elvehjem homogenizer. The homogenate is heated in a boiling water bath for 5 min; while still warm the pH of the homogenate is

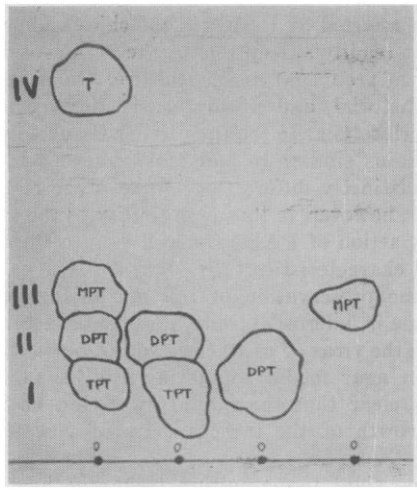

FIG. 1. Chromatogram obtained from solutions of 1 mM thiamine, monophosphothiamine, diphosphothiamine, and triphosphothiamine. Each spot corresponds approx to 10 μg of each compound.

brought to 6.2 by addition of 10% NaOH. After centrifugation at 3000 rpm for 5 min, the clear supernatant is brought to pH 4.5 by addition of 10% trichloracetic acid. Two g of fuller's earth is then added, and the mixture is stirred vigorously for 10 min. After centrifugation the supernatant is discarded and the fuller's earth is washed with a mixture of ethanol-ethyl ether (1:1) in order to eliminate the fatty substances. Thiamine and its esters are then eluted by stirring the fuller's earth with 3 ml of a mixture of pyridine-water-acetic acid (4:1:0.1) for 10 min. The elution fluid is first separated by centrifugation and then concentrated at room temperature *in vacuo* to approx 0.2 ml. One tenth of the concentrated fluid is used for ascending chromatography on strips of Whatman No. 1 filter paper (3.5 × 45 cm) and a pyridine-water-isobutyl alcohol mixture (2:5:3) as solvent. The solvent is allowed to rise to a height of 30 cm. The paper is then dried in a current of hot air

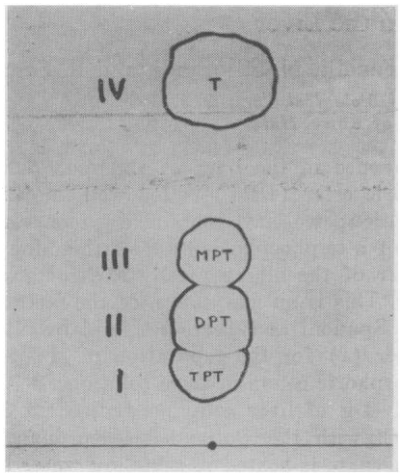

FIG. 2. Chromatogram obtained from 3 g rat liver.

and sprayed with a mixture of 55% ethanol, 10% NaOH, 2.5% $K_3Fe(CN)_6$ (5:5:0.1). Thiamine and its esters are in this way transformed into the corresponding thiochromes and will appear as fluorescent blue spots in ultraviolet light.

A typical chromatogram obtained from an aqueous solution of thiamine and its mono-, di-, triphosphoric esters is reproduced in Fig. 1. A chromatogram obtained from the liver of rat is presented in Fig. 2.

Figs. 1 and 2 reproduce only the areas of fluorescence. For approximate comparative evaluation of the single spots the intensity of fluorescence of the spots should also be taken into account. Spot No. 4 (thiamine) is generally less intense than other spots.

It will be seen that the four spots obtained in Fig. 2 correspond to those of Fig. 1. By addition of T, MPT, DPT, and TPT to the liver extracts the same four spots were obtained.

TABLE 1*

	Total P (μg)	Hydrolyzable P (μg)	Cocarboxylase activity ($CO_2 mm^3$)	Hydrolyzable P × 100 / Cocarboxylase activity ratio	Solubility in isobutyl alcohol
I Spot (TPT)	1.01	0.71	36	1.95	–
II Spot (DPT)	1.15	0.63	56	1.12	–
III Spot (MPT)	0.31	0	0	—	–
IV Spot (T)	0	0	0	—	+

* All the values are referred to 1 g of rat liver. (TPT = triphosphothiamine; DPT = diphosphothiamine; MPT = monophosphothiamine; T = thiamine.)

In order to identify the four compounds we have sought further evidence. Two chromatograms were run in parallel: one (A) was used to localize the spots; the other (B) was then placed upon A, and disks were cut from the paper in the position corresponding to the spots. The disks were eluted with water and the elution fluid was used for the following determinations:

1) The total phosphorus (*3*).

2) The hydrolyzable phosphorus in 2 *N* HCl at 100° for 10 min (by this treatment, according to Viscontini *et al.* (*4*) all the polyphosphoric esters of thiamine are hydrolized to MPT).

3) The cocarboxylase activity (*5*).

4) The ratio "hydrolyzable phosphorus/cocarboxylase activity." (Since TPT and DPT exert practically an identical enzymatic action, the ratio hydrolyzable phosphorus/cocarboxylase activity allows us to determine the number of hydrolyzable phosphoric radicals present in the molecules of the examined compound.)

5) The microbiological activity (*6*).

6) The solubility in isobutyl alcohol (*7*).

The evidence (Table 1) is as follows:

1) Spot I consists mainly of TPT, because the compound has a cocarboxylase activity and the hydrolyzable phosphorus/cocarboxylase activity ratio is practically

equal to 2. This means that for each enzymatically active molecule two labile phosphate radicals are present.

2) Spot II consists mainly of DPT, because the compound exerts a cocarboxylase activity and the hydrolyzable phosphorus/cocarboxylase activity ratio is practically equal to 1. This means that for each enzymatically active molecule one labile phosphate radical is present.

3) The compound present in Spot III can be identified with the MPT because, although inactive as cocarboxylase, it has a microbiological activity; it contains bound phosphate which is not split by 10 min hydrolysis; its thiochrome derivative is not soluble in isobutyl alcohol.

4) The compound present in Spot IV can be identified with thiamine because it does not contain phosphorus, is microbiologically active, and is readily soluble in isobutyl alcohol.

Researches on the presence of phosphoric polyesters of thiamine in other organs and on the enzymatic breakdown of these compounds will be published later.

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Comments and Communications

Plankton Terminology

THE term "ultraplankton" has been used recently (*1–3*) to describe the smallest known elements of the marine plankton—e.g., flagellates and green and blue-green algae, which are now generally recognized as being of primary importance as food for the majority of marine larvae (*1, 4, 5*). The use of this term has arisen from the need to differentiate the smaller members of the nannoplankton (or nanoplankton) from the great mass of larger flagellates, nonmotile algae, and peridinians that are too large to serve as food for the early larvae of worms, mollusks, crustaceans, etc.

To the writer the word "ultraplankton" is unacceptable, and it appears desirable to suggest an alternative before it gains general currency. "Ultraplankton" is of bastard Latin and Greek origin and, further, does not bear the meaning ascribed to it by its users. The prefix *ultra* is commonly translated as "beyond," as in ultramarine or ultraviolet, but the combination "ultraplankton" is practically meaningless. Nor is the term "μflagellates," as used by Scottish workers (*6, 7*), admissible, except colloquially, since there are many nonmotile algae present in company with flagellate forms.

It is suggested that the term "hekistoplankton" should be adopted (ἥκιδτος = "least") and should be defined as including all those elements in the plankton 10 μ or less in diameter of cell body. The limit 10 μ is suggested since the majority of larger forms cannot be ingested by marine larvae. It is in relation to their value as food for such larvae that these small elements in the plankton are principally studied; they apparently form the basis of the food chain in most marine planktonic communities.

In preparing this note I have had the benefit of advice on the choice of words of my colleague, M. N. Mistakidis, who has also drawn my attention to the frequent use of the prefix *macro* when *megalo* would be more appropriate. I understand that in modern usage "μακρος" is used to describe objects that are long rather than generally bulky, for which "μεγαλος" is more appropriate.

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The Green Peach Aphid on Tobacco in the Dominican Republic[1]

THE green peach aphid, *Myzus persicae* (Sulzer), first appeared in the United States in economically destructive numbers in 1946, in Gadsden Co., Fla. (*1, 2*). Ever since, its progression across the tobacco fields of the U. S. has brought forth various explanations for the phenomenon of its distribution. One explanation frequently advanced is that the development of infestations bears some correlation to the introduction of new synthetic insecticides, especially DDT, in and near the established tobacco-growing areas; it claims, in effect, that these insecticides destroy the natural predators of the aphids and that before these

[1] A contribution from a technical agricultural project in the Dominican Republic, operated jointly by the government of the republic and by the Office of Foreign Agricultural Relations, USDA. U. S. participation in this work is carried out as part of the Point IV program in the Dominican Republic, administered by the Technical Cooperation Administration, U. S. Department of State.