TABLE 1

HEMOGLOBIN LEVELS OF COBALT-DEFICIENT LAMBS TREATED WITH VITAMIN B_{12}

Sheep No.	B_{12} treatment	Dosage	Hemoglobin level (g/100 ml)					
			Pre-treatment	1st week	2nd week	3rd week	4th week	5th week
		(μg/week)						
1	Crystalline—injection	2	7.1	6.3	died			
17	Crystalline—injection	6	5.2	3.3	2.7	removed		
7	Crystalline—injection	9	4.4	6.0	5.6	5.7	6.0	6.2
9	Crystalline—injection	large*	6.0	4.9	6.0	4.9	4.9	4.9
7	Orally—concentrate	30	6.3	6.2	5.8	6.2†	6.6	6.6
7	Orally—concentrate	120	4.9	4.4	4.2	4.6†	5.7	6.6

* Single injection of 25 μg followed by another of 100 μg during second week.

† Initial dosage doubled.

TABLE 2

AVERAGE DAILY WEIGHT GAIN OF COBALT-DEFICIENT LAMBS TREATED WITH VITAMIN B_{12}

Sheep No.	B_{12} treatment	Dosage	Average daily gain (lb/day)					
			1st week	2nd week	3rd week	4th week	5th week	6th week
		(μg/week)						
1	Crystalline—injection	2	*	*	died			
17	Crystalline—injected	6	.34	*	*	removed		
7	Crystalline—injected	9	.54	.27	.13	.07	*	*
9	Crystalline—injection	large†	*	.06	.29	.33	*	*
7	Concentrate—orally	30	.03	*	.19‡	.29	*	.36
9	Concentrate—orally	120	*	.23	*‡	*	*	*

* Lost weight for the period.

† Single injection of 25 μg followed by another of 100 μg during second week.

‡ Initial dosage doubled.

tramuscularly twice per week during the period of treatment. Following the period of injections, two lambs were kept under study and fed vitamin B_{12} concentrate.[1]

The levels of vitamin B_{12} chosen for treatment were quite arbitrary, since we had little to guide us from the literature. West (*4*) reported favorable responses in pernicious anemia patients injected with single doses of 3.6 and 150 μg, indicating that the compound had high biological potency.

Results following vitamin B_{12} therapy, in terms of hemoglobin levels and weight gains in lambs, are summarized in Tables 1 and 2. It is noted that there was no significant response in these cobalt-deficient lambs when injected with crystalline vitamin B_{12} in amounts as high as 125 μg. The number of observations was necessarily small since the supply of vitamin B_{12} was very limited; however, the results were clearly negative. Neither was there a response in those lambs fed the vitamin B_{12} concentrate over a period of 6 weeks. Although the concentrate contained cobalt the amount was apparently too small to give a response to cobalt *per se*.

These preliminary and limited observations give no support to the theory that vitamin B_{12} is an important intermediary in cobalt metabolism in lambs.

[1] Supplied through the courtesy of Merck and Company.

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Synthesis of Tris(monofluorophenyl) methane and Tris (parafluorophenyl) methane

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In our search for a liquid dielectric, many aromatic fluorides were prepared in our laboratories, using the Balz-Schiemann (*1*) reaction. These compounds were also coupled by condensation reactions, or by Friedel and Crafts reactions, into symmetrical or unsymmetrical complex molecules.

The preparation of triphenyl methane from benzene and carbon tetrachloride with anhydrous aluminum chloride progressed with such ease and with such good yields (68–84%), that the Friedel and Crafts reaction was used to synthesize substituted triphenyl methane molecules with fluorine in the benzene rings.

Tris(monofluorophenyl) methane was synthesized by reacting 3.5 moles of monofluorobenzene and 1 mole of carbon tetrachloride with anhydrous aluminum chloride according to the procedure for the preparation of triphenyl methane as described and explained by J. F. Norris (*2*). The mechanism of the synthesis accordingly would be:

$$3\ C_6H_5F + CCl_4 + AlCl_3 \longrightarrow (C_6H_4F)_3CCl \cdot AlCl_3 + 3\ HCl$$

$$(C_6H_4F)_3CCl \cdot AlCl_3 + (C_2H_5)_2O \longrightarrow (C_6H_4F)_3CH + CH_3CHO + C_2H_5Cl + AlCl_3$$

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This reaction proceeded smoothly and the product was a liquid rather than a solid like triphenyl methane. The liquid was oily, water-white, and sweet-smelling. In reflected light it exhibited a violet fluorescence. It distilled over in a range of 200–210° C under 125 mm of pressure. The fact that it was a liquid was attributed to the possibility that it might be not a pure compound but a mixture of isomers. Further studies are being made on this point.

p-Difluorobenzene in a similar Friedel and Crafts reaction should form a pure compound and not a mixture of isomers. With the fluorine atoms in the para position in the benzene ring any linkage with the central methane carbon is ortho to one of the fluorine atoms and meta to the other. Since no other linkage is theoretically possible, a pure crystalline compound should result.

Using the same mole concentration as in the previous preparation but substituting *p*-difluorobenzene in the place of monofluorobenzene, the reaction progressed smoothly and gave an over-all yield of 45% of tris(*p*-difluorophenyl)methane. The product obtained was a yellowish crystalline solid. After three recrystallizations from 20 to 30% alcohol, pure white crystals were obtained. The mp of the pure crystals was 98.0–98.5° C.

Analysis (by H. S. Clark, Urbana, Illinois) for $C_{19}H_{10}F_6$:

	Calculated	*Found*
C	64.78%	64.94%
H	2.86%	2.86%
F	32.36%	32.20% (by difference)

The experimental details for these compounds will be reported shortly.

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Measuring the Thickness of Very Thin Microtome Sections

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Several authors have recently described methods of sectioning biological material for electron microscopy. Some have used a high speed microtome (*3*, *4*), others a microtome at ordinary speed with various modifications (*1*, *2*, *5*, *7*). It seems desirable to have sections at least as thin as 0.1 μ, while standard methods result in sections only as thin as 2 μ, or at best 1 μ.

During the war the writer developed a method for cutting some materials consistently at 0.05 μ and even less. Examination of these sections with the electron microscope failed, in the writer's opinion, to show the desired detail in the chromosome material in which he was interested, and so the work was discontinued. The method consists essentially in the use of: a purified and very hard wax (carnauba) at a low temperature, a specially constructed microtome of the rocker type at ordinary speed, and a beryllium bronze blade. Beryllium bronze is not very hard (about Brinell 380) but its texture is very uniform and it is easy to secure an extremely even and sharp edge. While the method did not succeed in the purpose for which it was intended, it might prove helpful with other materials. The author hopes to publish a full description of it soon. During this work, a simple and accurate means was found for measuring the thickness of the sections. As this may be generally useful, it is described herein.

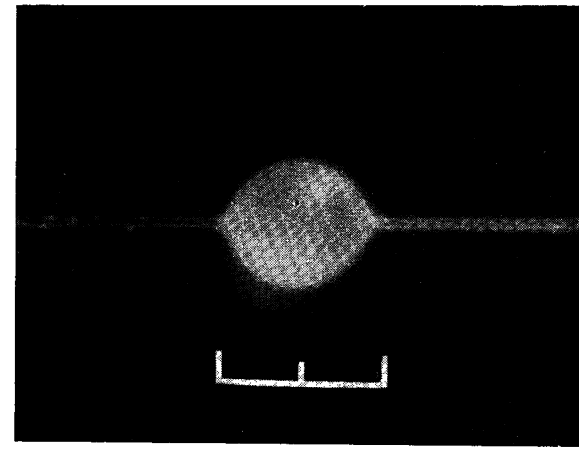

Fig. 1. A sphere of wax resulting from the melting of a section on a thin glass fiber. The length of the scale is 0.1 mm.

Several optical methods are available for measuring thin films (*6*, *8*), but their use is difficult when the area of the section is only of the order of a square millimeter. A much simpler way is to measure under the microscope the area of the section, then to melt the section into a sphere from whose diameter the volume of wax can be calculated (Fig. 1). To obtain the sphere, the section is caught on a very thin (less than 10 μ) glass fiber, and the fiber is mounted in the field of a binocular. A small loop of electrically heated wire is carefully guided close to the section, which is slowly melted. If the glass fiber is thin enough, an almost perfect single sphere will result; for greater accuracy one may prefer to calculate its volume as an ellipsoid. It is important to watch the heating under fairly high magnification, and to heat slowly, as too high a temperature will cause evaporation.

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