mal human beings who were between 20 and 40 years of age. Each serum was tested immediately as it was removed from the clot, and again after it had been heated at 56° C for 30 min. The hyaluronidases used in these tests were unrefined filtrates of cultures of type 3 *Pneumococcus*, hemolytic *Staphylococcus aureus*, *Clostridium perfringens*,[1] beta hemolytic *Streptococcus* (group A),[2] and purified bovine testicular hyaluronidase. Twofold serial dilutions of each serum were titrated against the constant strength of each enzyme. Hyaluronidase inhibition was measured by the mucoprotein clot prevention test as previously described (*7*), except that fresh egg albumin was used instead of normal horse serum as the protein component of the substrate. With this modification of the test, a fourfold variation of serum inhibition titer could occur by chance, so that eightfold variation of titer was considered significant. If a 1: 3 dilution of a serum did not inhibit an enzyme, inhibitor was considered to be absent from the serum. Hyaluronidase inhibition by serum was considered thermostable if heating the serum caused no significant fall in the titer.

Before heating the sera at 56° C for 30 min, all inhibition of the five hyaluronidases by normal sera occurred in a serum dilution of 1: 48 or less, except that two sera in dilution of 1: 192 and four sera in dilution of 1: 96 inhibited the pneumococcus hyaluronidase, one serum in dilution of 1: 384 inhibited the staphylococcus hyaluronidase, and one serum in dilution of 1: 96 inhibited the streptococcus hyaluronidase. Results tabulated below reveal thermostable inhibition of the five hyaluronidases by normal sera:

TABLE 1

Hyaluronidase tested	No. of sera tested	No. of inhibiting sera before heating	No. of thermostable inhibiting sera
Pneumococcus	50	47	45
Staphylococcus	49	23	19
Cl. perfringens	50	16	10
Streptococcus	50	12	7
Testicular	48	46	2

Tests upon consecutive daily sera of four persons revealed consistent inhibition of the pneumococcus, staphylococcus, and testicular hyaluronidases. However, there was day-to-day fluctuation in the inhibition of the streptococcus and *Cl. perfringens* hyaluronidases, so that results of the inhibition of these two enzymes tabulated above are of undetermined immunological significance.

A further preliminary investigation of the thermolabile serum inhibition of *Cl. perfringens*, streptococcus, and testicular hyaluronidases was carried out. After this inhibition had been destroyed by heating the sera at 56° C for 30 min, it was completely restored in most sera and partially restored in remaining sera by the addition of complement (*5*). The complement used was 0.5 cc of a 1: 30 dilution of normal guinea pig serum, which alone did not inhibit the test strength of the enzymes.

[1] This hyaluronidase was glycerol-dialyzed. It was prepared and supplied by Dr. A. A. Tytell, Cincinnati, Ohio.

[2] Culture supplied by Dr. R. M. Pike, Dallas, Texas.

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Vitamin B_{12} and Cobalt Deficiency in Sheep

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The discovery by Rickes *et al.* (*2*) that vitamin B_{12} is a cobalt complex pointed to the possibility that this vitamin was an intermediary in the metabolism of cobalt in those species requiring this element. It has been suggested by several workers that cobalt, which is known to be required by ruminant but not by nonruminant animals, functions primarily through some unknown mechanism in the rumen, probably related to the microflora. This theory enjoys some but not conclusive experimental support.

At the time that the report of Rickes *et al.* appeared, we were engaged in studies of cobalt deficiency in lambs. Among the symptoms displayed by these lambs were loss of appetite, anemia, loss in body weight, and eventual death. Such symptoms have previously been reviewed by Russell (*3*). Among the treatments under study were the effects of cobalt administration by feeding vs. injection. It was observed that deficient lambs when fed 1 mg of cobalt per day responded quickly in improved appetite, gains in weight, and increase in hemoglobin concentration of the blood. On the other hand, deficient lambs when injected with the same quantity of cobalt showed no detectable response over a period of 7 weeks. It thus seemed possible, assuming that vitamin B_{12} is a necessary metabolite for sheep, that cobalt orally administered may be synthesized into vitamin B_{12} by the rumen flora; and that cobalt injected was incapable of this transformation, possibly because insufficient quantities of it reached the rumen Comar *et al.* (*1*). According to this postulate cobalt deficiency is essentially a vitamin B_{12} deficiency and if this vitamin is furnished the deficient animal directly by injection, a favorable response should follow. Since cobalt salts injected into deficient lambs gave no response it seemed safe to assume that the much smaller amount of cobalt present in vitamin B_{12} would not give a response *per se*.

Cobalt-deficient lambs which had previously been injected with cobalt salts were chosen for this study. All of these lambs had pronounced symptoms of cobalt deciency. They were injected with various amounts of crystalline vitamin B_{12}[1]. The injections were made in-

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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