TABLE 1

EFFECT OF VITAMIN B_{12}, VITAMIN C, AND LEUCOVORIN ON THE THYMUS GLAND IN PGA DEFICIENT CHICKS INFECTED WITH *Ascaridia galli*

Expt. no.	Diet	No. animals		Thymus weight
		Start	Finish	
5	Basal (no PGA)	27	7	.063
	Basal + 200 μg PGA/100 g diet	14	14	.513
7	Basal (no PGA, no B_{12})	25	10	.104
	Basal + 5 μg B_{12}/100 g diet	24	8	.089
	Basal + 100 mg vitamin C/100 g diet	24	22	.088
	Basal + B_{12} + vitamin C	21	7	.197
	Basal + B_{12} + vitamin C + PGA	24	23	.597
10	Basal (no PGA, no B_{12})	20	7	.069
	Basal + B_{12}	21	11	.131
	Basal + PGA	18	18	.380
	Basal + PGA + B_{12}	14	14	.543
	Basal + 400 μg leucovorin/100 g diet	18	18	.531

it would seem that Sadun was actually working with a deficiency of both PGA and vitamin B_{12}. From this one might also assume that his control birds which were given adequate PGA were still deficient in vitamin B_{12}. Closely related to the biological activity of vitamin B_{12} and PGA is leucovorin and vitamin C. It is currently believed that leucovorin is the active form of PGA (*2*), and that vitamin B_{12} participates in the formation of leucovorin from PGA in chicks (*3*). Vitamin C on the other hand appears to be synergistic in nature. Dietrich *et al.* (*4*) reported that vitamin C enhanced vitamin B_{12} activity and both vitamin C and vitamin B_{12} stimulated *in vivo* synthesis of PGA.

In view of these intimate metabolic interrelations, studies were initiated to ascertain the effect of PGA, vitamin B_{12}, vitamin C, and leucovorin on *A. galli* infections and on the thymus gland of infected chicks. The action of these compounds on *A. galli* infections as well as complete information as to techniques employed and composition of the basal ration will be reported elsewhere (*5*). Day-old white leghorn chicks, obtained from a commercial hatchery, were used in all experiments. Chicks were infected at 2 wk of age and 3 wk later were autopsied. Thymus weights were obtained using a Roller-Smith balance and recorded as thymus weight/100 g body weight.

The dramatic atrophy of the thymus gland in the absence of PGA (Expt. 5) could be used to elucidate the interactions of PGA with other compounds. The addition of vitamin B_{12} (5 μg/100 g diet) to a basal diet deficient in both PGA and vitamin B_{12} had no consistent influence on thymic growth. Likewise the addition of vitamin C (100 mg/100 g diet) to this basal diet did not stimulate the growth of the thymus gland. However, the addition of both vitamin C and vitamin B_{12} did increase the thymus weight but did not approach the level produced by the addition of PGA (200 μg/100 g diet) to the diet (Expt. 7).

On the other hand, the relative weight of the thymus gland in the presence of leucovorin (400 μg/100 g diet) is almost equal to the weight level of the thymus gland in the presence of both PGA and vitamin B_{12} (Expt. 10). The weight of the thymus gland in the presence of leucovorin is significantly greater than the weight produced by PGA alone, or by vitamin B_{12}.

As had been mentioned earlier, vitamin B_{12} has no consistent effect on the thymus gland in the absence of PGA, but in the presence of PGA, vitamin B_{12} significantly increased the weight of the thymus gland equal to the weight produced by leucovorin alone. Limited numbers of noninfected control chicks were run simultaneously in all 3 experiments with the same results reported herein. Using the weight of the thymus gland in chicks as a criterion, evidence is presented to support the concept that leucovorin is the active form of PGA and that leucovorin is biologically equivalent to PGA plus vitamin B_{12}.

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Report of a Second Example of the Rh Agglutinogen c^v, with Some Comments on Its Relation to the Agglutinogen f [1]

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The recent discovery of a new member of the Rh family of agglutinogens known as f (*1*) has raised several issues both of practical and theoretical importance. In a previous communication (*2*) the present authors reviewed the genetic theories which would most adequately explain the inheritance of the new factor and also made the suggestion that the antigen f might possibly be determined by a "position effect" (*3*) of the genes *c* and *e*. Essentially it was proposed that the presence on one chromosome of the gene combination *ce* would give rise to the f antigen in red cells. Replacement of either *c* or *e* by the alleles *C* or *E*, respectively, would destroy the position effect, and the f antigen would accordingly be absent from the cells. It was predicted that the replacement of the genes *c* or *e* by alleles which caused even minor modification of the corresponding cell antigens might be

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as effective in destroying the position effect as alleles which caused a major change in these cell antigens.

Recently a series of blood samples have been obtained from a family of unusual genetic composition. In this family it was possible to follow the transmission of an allele of *c* (probably c^v) through three generations and to demonstrate that the association of this allele on the same chromosome with *e* did not give rise to the antigen f.

After delivery of an erythroblastotic fetus the serum of the mother was found to contain an anti-Rh antibody with the specificity anti-D. This was her first pregnancy, but inquiry revealed the fact that she had received an intramuscular injection of her father's (Rh positive) blood about 18 yr previously. Other examples of this unfortunate situation have been described by Levine (*4*).

The blood of her husband (the propositus) was typed in an attempt to determine zygosity at the *Dd* locus, but since the results were not conclusive all other available relatives of the husband were typed with the 5 usual anti-Rh sera and also with anti-C^w and the new antibody anti-f. The results obtained from this investigation are in Table 1. It can be seen that

TABLE 1

REACTIONS OF BLOODS OF FAMILY UNDER INVESTIGATION WITH FIVE USUAL ANTI-Rh ANTISERA AND ALSO WITH ANTI-C^w AND ANTI-f

Spec. No.	Relation to propositus	Reaction of blood with Rh antisera						
		anti-C	anti-C^w	anti-D*	anti-E	anti-c	anti-e	anti-f
1	Father	+	0	++++	++++	++++	+++	0
2	Mother	++++	0	++++	0	0	+++	0
3	Brother	++++	0	++++	0†	++++	+++	0
4	Propositus	++++	0	++++	0†	++++	+++	0
5	Wife	0	0	0	0†	++++	+++	+++
6	Child	+	0	++++	0†	++++	+++	+++

Note. The symbols ++++ and +++ indicate strong reactions. A weak but definite reaction is indicated by the symbol +, whereas a negative reaction is expressed as 0.

* Each specimen was tested with 2 different examples of anti-D, the negative result of specimen 5 being confirmed by the indirect Coombs' technic.

† These specimens were tested with 4 different examples of anti-E.

the father (specimen 1) of the propositus as well as the child (specimen 6) gave a weak reaction with the anti-C serum.[3] This phenomenon suggested that the antigen determined by the *Cc* locus might in these individuals be due to an allele such as C^u or c^v (*5*). These two bloods were therefore investigated more fully from this point of view. Each specimen was tested with 7 different anti-C sera in addition to the one already used in the routine typing.

Table 2 gives the reactions obtained by this procedure together with the results of testing control *Cde/cde* and *cDe/cde* cells in parallel. The results established the fact that these two bloods contained

[3] Experience with this serum has shown it to give very strong reactions with "normal" C antigens.

TABLE 2

REACTIONS OF BLOODS FROM FATHER OF PROPOSITUS (#1) AND CHILD OF PROPOSITUS (#6) WITH 7 ANTI-C SERA

(Control positive cells were of type *Cde/cde*; control negative cells were of type *cDe/cde*)

Blood sample	Anti-C sera*						
	R-39	3149	And.	Ada.	101b	Bas.	Jac.
Family blood #1	±	++	0	0	+	0	+++
Family blood #6	+	++	0	0	+	±	+++
Control positive	++++	+++	+++	+++	++++	++++	++++
Control negative	0	0	0	0	0	0	0

Note. The Symbols ++++, +++, ++, +, ±, 0 represent varying strengths of agglutination from maximum strength through weaker grades to complete absence of clumping.

* These seven anti-C sera are different from each other and from the one previously used in routine typing.

an antigen which reacted with some, but not all, anti-C sera. This antigen was tentatively classified as C^u or c^v. This distinction between these 2 antigens depends on the demonstration of a "dosage" effect, it being known that the cells of an individual with the genotype cc^v behave as though they possessed 2 units of the antigen c while the cells of a person with the genotype C^uc behave as though they possessed a single unit of c. A "dosage" titration was therefore set up in the manner recommended by Race and Sanger (*6*), using two different anti-c sera and assigning "scores" to the various strengths of agglutination. In these comparative titrations samples 1 and 6 from the family under investigation clearly showed the double dose effect (Race and Sanger score 50), behaving more like the *cde/cde* control blood (Race and Sanger score 48) than like the control *CDe/cde* blood (Race and Sanger score 35). The C variant present in these individuals thus behaved like the variant c^v of Race, Sanger, and Lawler (*5*) rather than their variant C^u. It now became possible to construct an accurate genealogy of the Rh chromosomes in this family, as shown in Fig. 1.

The criteria defining genes allelomorphic to *C* were laid down by Race, Sanger, and Lawyer in 1948 (*5*). The family described by us represents the second example of c^v and is the first demonstration of its transmission through three generations.

It is of interest to explore the relationship of the allele c^v to the antigen f. It was originally shown by Rosenfield *et al.* (*1*) and confirmed by the present authors (*2*) that there is a very close association between the genes *c* and *e* and the antigen f. The nature of this association was such that the antigen f was not present in the red cells unless an individual possessed a chromosome bearing the genes *c* and *e* (that is, the chromosomes *cde* and *cDe* or, more strictly positionally, *dce* and *Dce*). The association with the *ce* gene combination, while close, was found not to be invariable, 3 examples out of a total of 35 *CDe/cde* random bloods being f negative in our series. The antigen f has not been detected up to the present time in the absence of the *ce* gene combination.

Rosenfield *et al.* suggested that the inheritance of the f antigen could be explained by the postulation of a pair of allelic genes *Ff* closely linked to the pairs *Cc, Dd,* and *Ee.* Chromosomes known to confer the antigen f on the cells would therefore be *cdef* and *cDef*, while typical examples of chromosomes not associated with the presence of f would be *CDeF, cDEF, CdeF,* etc. Implications of this hypothesis are that there should exist an antigen F and a corresponding antibody anti-F; also it would be expected that chromosomes such as *cdeF* and *CDef* would exist. The

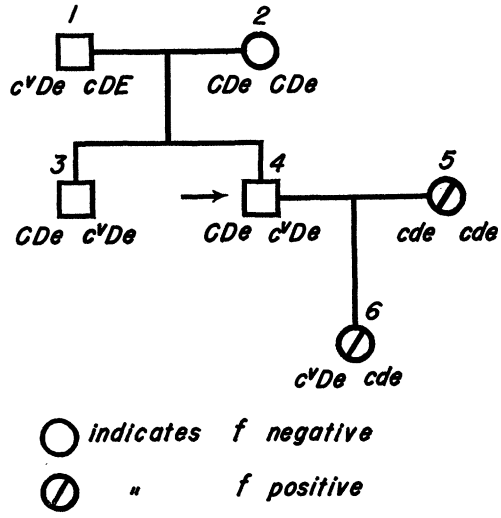

FIG. 1.

finding of *cde* chromosomes which were not associated with the antigen f supports in some measure the fourth locus hypothesis, these chromosomes presumably being *cdeF*. The failure to find chromosomes such as *CDef* and *cDEf* does not detract at this stage from the hypothesis, since it can be presumed that these chromosomes are rare.

The present authors suggested that another possible explanation of the data "would consider the f antigen to be the result of a 'position effect.' This hypothesis would state that when the genes *c* and *e* are together on the same chromosome the antigen f is present in the red cells" (*2*). It was further postulated that the "exceptions," that is, instances in which *c* and *e* are apparently on the same chromosome and the antigen f is absent, are due to the presence of alleles of *c* or *e* which fail to interact to produce f. The "position effect" hypothesis suffers the defect that it can be proven only if a crossover occurs in which the genes *c* and *e* are separated and at the same time the f antigen is lost. Such a crossover would simultaneously prove the truth of the Fisher-Race hypothesis of the inheritance of Rh antigens. The discovery of an anti-F serum would immediately invalidate the "position effect" hypothesis, as would the discovery of the f antigen in the absence of the genes *c* or *e*.

The family reported in this study is an example of a situation predicted by the "position effect" theory. In this family there are three individuals (the propositus, his father, and his brother) who are f negative and who possess the chromosome c^vDe. The daughter of the propositus who is f positive also possesses the chromosome c^vDe, but it is apparent that her f antigen is derived from the *cde* chromosome which she inherited from her mother.

The data provided by the analysis of the bloods of this family lend support to the "position effect" hypothesis, although they do not contradict the postulation of a fourth locus in the Rh complex; nor, for those who favor it, do they invalidate an extension of the multiple allele hypothesis.

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Fatty Acid Absorption and Chylomicrons

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The absorption pathway of long-chain fatty acids is still controversial. Frazer (*1, 2*), working with rats, found that ingested oleic acid did not give rise to chylomicronemia and, therefore, reasoned that the absorption pathway of the fatty acid was through the portal system. However, Bergstrom *et al.* (*3*), Bloom *et al.* (*4*), Reisner (*5*), and Tidwell (*6*), have been unable to confirm this view.

Our attention was drawn to this controversy because of our interest in the effect of bile salt on lipid absorption. In a previous communication (*7*), it was shown that a neutral fat would give rise to chylomicronemia when absorbed from a Thiry fistula that contained no bile or pancreatic secretion, implying that neither was necessary for particulate fat absorption. If it could be shown that a fatty acid does give rise to chylomicronemia when placed into a Thiry fistula, a similar interpretation as with the neutral fat experiments could be made.

To study oleic acid absorption, 3 different types of experiments on dogs were designed. In the first, the fatty acid was ingested by the animals; in the second, the acid was placed into a jejunal Thiry fistula; and in the last, the fatty acids with a bile salt were put into a Thiry fistula.

The 3 Thiry jejunal fistula dogs were the same animals studied previously (*7*). In all experiments, 10 ml of c.p. oleic acid was used, as this amount of neutral fat has been shown to give rise to a significant chylo-

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