the sulfonamide. Four per cent methyl urea was bacteriostatic when used alone and any stimulation of the activity of sulfanilamide by it could, therefore, not be determined.

II. *The Effect of Methyl Urea on Para-aminobenzoic Acid.* A 2% concentration of methyl carbamate did not inactivate *p*-aminobenzoic acid. Four per cent was moderately effective, the sulfonamide-inhibiting substance being inactivated in amounts as large as 0.1 mg per 100 ml for 24 hours. As with urea and urethane, the anti-PABA activity of methyl urea appeared to be of short duration.

III. *The Bacteriostatic Effects of Thiourea.* A 2% concentration of thiourea inhibited growth of *S. aureus* for 96 hours, 1% for only 48 hours. Quantities less than 1% were ineffective. One per cent thiourea suppressed completely the multiplication of *E. coli.*

Summary and Discussion. Methyl urea was found to be bacteriostatic for several Gram-negative bacteria in both VIB-S and in a synthetic medium. The concentration of drug required to suppress growth varied somewhat with each organism, but it ranged generally between 6 and 8% except for *Ps. aeruginosa,* which was inhibited by a 4% solution. The activity of the drug in a synthetic medium was greater than in VIB-S.

The substitution of one $—NH_2$ in carbamide by a single $—CH_3$ group increased the activity of urea very little since approximately the same concentrations of the 2 compounds were required to inhibit the growth of bacteria. Urethane, as previously reported, was more highly bacteriostatic than either urea or methyl urea. It would appear, therefore, that the insertion of $—CH_2CH_3$ in place of one $—NH_2$ in urea results in greater activity than that following the addition of only a single carbon atom. As shown by the few data presented, the replacement of $=O$ by $—S$ in the urea molecule is apparently very effective in increasing its antibacterial properties since a 1% concentration of thiourea produced approximately the same results with *E. coli* as 3% urethane or 6% urea.

Conclusions. 1. Methyl- and thiourea are bacteriostatic for Gram-negative bacteria. 2. The substitution of an $—NH_2$ group by $—CH_3$ in urea results in no demonstrable increase in antibacterial activity. 3. The replacement of the $=O$ group in urea by $—S$ increases markedly the antibacterial activity. 4. Methyl urea, in the concentrations studied, does not potentiate the sulfonamides. It does have a moderate antagonistic effect on *p*-aminobenzoic acid.

15653

Interrelation Between α-Tocopherol and Protein Metabolism: Body Weight and Tooth Pigmentation of Rats.*

E. L. HOVE. (Introduced by Philip L. Harris.)

From the Research Laboratories of Distillation Products, Inc., Rochester, N. Y.

A relation between vitamin E and protein metabolism has been suggested in 3 reports in the literature. Cerecedo and Vinson[1] observed that a 58% litter incidence of weanling paralysis in mice was reduced to 28% by increasing the casein in the diet of the mothers.

Dam[2] noted that when rats were placed upon a low-protein diet, the group receiving α-tocopherol survived somewhat longer than the E-free controls. However, the *rate* of body weight loss was the same in the 2 groups, and the E-fed group had a lower

* Communication No. 106.

1 Cerecedo, L. R., and Vinson, L. J., *Fed. Proc.*, 1944, **3**, 55.

2 Dam, H., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 55.

body weight at death. Victor and Pappenheimer,[3] on the other hand, reported a very marked effect of tocopherol in conserving the body weight of rats on a low-casein diet. This work is complicated by the fact that a cirrhosis-producing diet (low choline as well as low casein) was used. The effect of tocopherol possibly could have been related to the altered metabolism in the cirrhotic state.

Since these 2 reports appear to be contradictory in some respects, we have repeated the work of Victor and Pappenheimer using equalized feeding of a diet low in casein but otherwise nutritionally adequate.

Fifty-four male weanling rats from the stock colony were placed upon a vitamin E-free diet of the following composition: crude casein, 22.5; sucrose, 63; salt mixture USP No. 2, 4.5; and lard, 10. B-complex vitamins were added to the casein to furnish 10 γ of thiamine, riboflavin and pyridoxine, 25 γ calcium pantothenate, 100 γ niacin, 1 mg of choline chloride, 0.1 mg *i*-inositol, and 5 γ vitamin K per g of ration. Each rat was supplemented weekly with 2,000 units vitamin A and 20 units of vitamin D in olive oil solution.

The animals were kept on this diet, *ad libitum,* until they reached 225 g in body weight. This required 5 to 7 weeks. As they attained this weight the rats were placed on a fixed daily amount of one of 3 diets which differed only in their casein content. These diets contained 5, 20, or 40% crude casein, and, except for compensatory changes in sucrose, were identical in composition with the diet described above.

A daily allotment of 8.9 g of the diet was fed to each rat in the morning and was generally consumed within an hour. The diets were made fresh weekly and kept refrigerated at all times. Half of the rats on each diet were supplemented with daily doses of 1 mg of d, α-tocopherol in olive oil solution.

The average body weight changes of the rats on the 5% casein diet (8.9 g daily) are shown in Fig. 1. These animals lost about 40 g in body weight during the first 4 weeks, after which the curves levelled off. α-Tocopherol had little influence during this period of adjustment to the new diet. Beginning at about the 10th week the vitamin E-low rats began a rapid weight loss. They continued to consume their daily allotment of food in spite of declining body weight. Their general appearance deteriorated rapidly. They became listless and showed marked signs of atony and muscular dystrophy. During the last 3 weeks on experiment 3 of the 9 animals regularly left up to 3 g of their diet unconsumed.

In sharp contrast to the vitamin E-low group, the animals receiving α-tocopherol regained some of their initial weight loss and thereafter maintained their weight. In spite of the low-protein intake their appearance was good. They were alert and showed normal general muscular tone. Statistically the difference in body weight between the E-low and E-fed groups was highly significant. At 21 weeks on experiment the standard error for the average weight of both groups was ± 3.5; this corresponds with a t value of

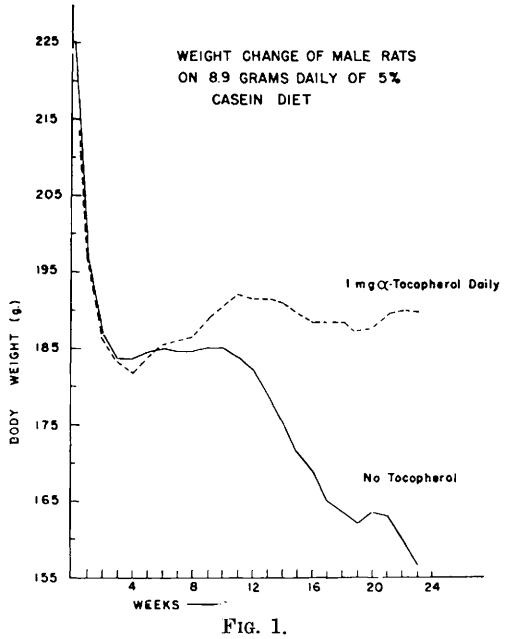

FIG. 1.

The effect of α-tocopherol on body weight changes of adult male rats shifted to a 5% crude casein diet.

[3] Victor, J., and Pappenheimer, A. M., *J. Exp. Med.*, 1945, **82**, 375.

6.2, while for P (0.01) the theoretical t value is 2.95.

At autopsy no signs of liver cirrhosis were evident in either group. The gross appearance and fat content of the livers were normal. Very marked atrophy of the testes was seen in the E-free group; their average testes weight was 0.4 g as compared with an average of 1.2 g in the E-fed group. Eight of the 9 low-E rats had marked hyperplastic crater lesions in the rumen of the stomach, while only 3 of the E-fed rats showed such lesions, and these were mild.

The animals on the higher protein diets required about 2 weeks to adjust themselves to the fixed limited quantity of the new diets. They lost weight during this period. However, after this time they resumed their growth. At the end of 24 weeks the group on the 20% casein diet averaged 292 g while the corresponding E-fed group averaged 303 g. On the 40% casein diet, the average weights were 298 and 308 g for the E-low and E-fed groups respectively. The animals all appeared normal. No signs of dystrophy were yet evident.

An interesting incidental observation has been made on tooth pigmentation in these rats. Granados and Dam[4] have shown that a combination of high-fat and low-E leads to loss of the normal tooth pigment. Our experiment has added protein to the factors influencing this yellow-orange color of the teeth. Table I shows the average color index of the maxillary incisors of the various groups after 10 and 20 weeks on the fixed daily intake of the diets. The color index was rated on an arbitrary scale with 5 degrees ranging from "0" to "4." All of the tocopherol-fed animals had essentially normal tooth color, regardless of protein content of the diet. However, it is clear that the amount of casein greatly influenced the tooth color in the non-tocopherol groups.

TABLE I.
Effects of α-Tocopherol and Protein on Tooth Pigmentation of Adult Rats Fed 8.9 g of Diet Daily. The values represent the average color ratings (0 to 4) of the groups.

Casein content of diet (%)	No tocopherol		Plus tocopherol	
	10th wk	20th wk	10th wk	20th wk
5	0.1	0	3.2	2.8
20	1.4	0.9	3.3	3.2
40	1.6	1.1	3.6	3.2

Discussion. Under the conditions of this experiment α-tocopherol was essential to the maintenance of body weight and general well-being of adult rats on a low-casein diet. The daily food intake was fixed at a level which, with a normal protein percentage, allowed continued growth and well-being even without tocopherol.

This may indicate that α-tocopherol "spares" or allows better utilization of some constituent of the casein. Conversely, the results may mean that low casein (or some constituent) greatly increases the requirement of the rat for vitamin E. The second explanation appears the more plausible and is currently being checked with rabbits as the experimental animal. Several pure amino acids have been added to low-casein diets at a 1% level in an attempt to find some specific factor which would counteract the dystrophy-inducing property of the low-casein diet. No benefit has been observed with tryptophane, valine, arginine, or lysine. A questionable benefit was seen with *l*-cystine. This work is being continued.

In the work of Dam[2] tocopherol apparently had no influence on the loss in body weight of rats on diets in which the only protein was that furnished by 10% yeast. However, in our work as well as that of Victor and Pappenheimer[3] tocopherol had a marked influence when the only protein was 5% crude casein. The difference in composition of casein and yeast protein may, therefore, furnish a clue as to a specific factor involved in vitamin E metabolism.

Summary. Adult male rats have been fed fixed daily quantities of vitamin E-low diets containing 5, 20, or 40% crude casein. On the 5% casein diet the animals lost weight and developed muscle dystrophy, testicular atrophy, stomach ulcers, and tooth depigmentation.

These symptoms of a vitamin E deficiency were prevented either by α-tocopherol or by

[4] Granados, H., and Dam, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 295.

the higher protein diets. The possible significance of a lack of individual amino acids in increasing the vitamin E requirement of rats is discussed.

15654

Induced Ovipositions in Relation to Age of Oviducal Egg in the Domestic Hen.

IRVING ROTHCHILD AND R. M. FRAPS.

From the Bureau of Animal Industry, U. S. Department of Agriculture, Agricultural Research Center, Beltsville, Md.

The removal of the ruptured follicle from the ovary of the hen results in a delay in the time of lay of the egg which arose from that follicle.[1] The experiments reported here are an approach to the problem of how the ruptured follicle functions to determine the time of lay. Specifically, they were designed to determine whether the sensitivity of the chickens' uterus to an oxytocic agent increases as the time of normally expected lay approaches.

Methods and Materials. Five groups of laying hens were injected intravenously with the same dose of an oxytocic agent at various hours after the ovulation of an egg. The results were expressed as the percentage of birds showing premature ovipositions.

Birds selected for injection carried in their oviducts the second egg of a 2-egg clutch, the first egg of which was laid at the recorded hour of either 10:00 a. m. or 11:00 a. m. The time of lay of all eggs in this laboratory is recorded on the hour every hour between 8:00 a. m. and 4:00 p. m., so that there was a maximum spread of no more than 2 hours between the time of lay of the earliest and latest first egg of a clutch in any group. Of all the birds used, those selected for 10:00 a. m. lay of the first egg of a clutch comprised between 55 and 60% of the total group in 4 groups and 74% of the total in the fifth group.

The presence of the oviducal egg was determined by digital palpation through the rectum. The age of the oviducal egg at the time of injection was estimated from the relationships between time of lay and ovulation of intraclutch eggs,[2,3] and the time required for the egg to pass through the various portions of the oviduct.[2,3] For 2-egg clutches, the interval between lay of the first egg and ovulation of the second is about 45 minutes,[2,3] and the time required for the egg to reach the uterus, about 4 hours from the time it enters the oviduct.[2]

The injections were made during the interval between ovulation and the time of expected lay (a period of about 27 hours) at the following hours: 4:00 p. m., 8:00 p. m., midnight, 6:00 a. m. and 10:00 a. m. Usually, no more than one minute elapsed between the injection of one bird and the injection of the next in each group; the maximum difference between the given times of injection and the time of the earliest or latest injection in the group was 34 minutes. White Leghorn and Rhode Island Red hens were used: the number of one breed did not exceed the number of the other by more than one bird in each group, except in that injected at 6:00 a. m. In this group there were 33 White Leghorns and 12 Rhode Island Reds. The results for the groups as a whole were not appreciably different from the results obtained in each breed.

The agent used to induce premature oviposition was Parke, Davis & Co. "Pitressin." Although the main constituent of this preparation is the pressor principle of the posterior

1 Rothchild, I., and Fraps, R. M., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 79.

2 Phillips, R. E., and Warren, D. C., *J. Exp. Zool.*, 1937, **76**, 117.

3 Unpublished data from this laboratory.