plete protection. Olive oil has been less efficacious. Fractionation of these oils is now being carried out in the attempt to gain further knowledge as to the nature of the protective factor.

7317 C

Experiments on Vitamin G Concentration and Possible Supplementary Relationships with the Vitamin G Deficient Diet.

J. WALLACE PAGE, JR. (Introduced by H. C. Sherman.)

From the Department of Chemistry, Columbia University.

Attempts to concentrate the vitamin G of food materials[1, 2, 3] before completion of the work here reported, have been complicated by the postulation of a multiplicity of factors in vitamin G.[4–14] While the question of this possible multiple nature of the vitamin still remains very unsettled, much progress has been made in the concentration of the vitamin.[15, 16, 17] The present paper reports additional evidence of the existence of some unknown variant in vitamin G research and describes a method of concentration of vitamin G which is being used as an initial procedure in the subsequent concentration work of Booher.[17]

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The method used in the evaluation of various materials for their vitamin G content was essentially that of Bourquin and Sherman.[18] During this investigation the small anti-coprophagy harness previously described[19] was first used to minimize the error due to coprophagy. In a group of 37 animals, half of which were harnessed, the average total gain at the end of the fifth week for the group restricted in respect to coprophagy was 18.8 ± 1.2 gm., while in the parallel group, unrestricted in respect to coprophagy, the corresponding gain was 29.4 ± 2.2 gm. (Deviation measure = P.E.). Observed activity of the animals and the greater precision in growth records of the harnessed animals indicates that the harness *per se* did not restrict growth.

Concentration Experiments. A concentrate of vitamin G from dried skimmed milk powder was obtained as follows: 500 gm. of air dried skimmed milk powder were refluxed successively and with constant agitation by means of a stream of purified nitrogen with 2000 cc., 1250 cc., and 750 cc. of neutral boiling 93-94% ethyl alcohol (by weight) for 30, 30, and 15 minutes respectively. The 3 alcoholic extracts combined, dried and analyzed showed a nitrogen and phosphorus content of 4.44 ± 0.02% and 0.47 ± 0.01% respectively (precision measure ± A.D.). The potencies of the alcoholic extract and of the extracted milk powder were measured and the results summarized in Table I (a, b, c). The alcoholic extract (5% of the solids of the original milk powder) was found to be 10 times as rich in vitamin G as the original milk powder. Half of the total activity was in the alcoholic extract and half in the extracted milk powder.

Evidence of Some Variant in Vitamin G Testing. A further attempt was made to concentrate the vitamin by reducing the alcoholic extract to a small volume by distillation under reduced pressure and adding to this concentrated extract an equal volume of ethyl ether. A precipitate was obtained (analysis: nitrogen = 3.49 ± 0.05%, phosphorus = 0.255 ± 0.005%; precision measure = A.D.) which was only about 4 times as rich in vitamin G as the original milk powder, Table I(d); whereas the alcoholic extract had shown a ten-fold concentration. The loss of activity could not be accounted for in the alcoholic filtrate remaining after ether precipitation. Attempts to ascertain any supplementary relation (evidence of vitamin G "factors") between the ether precipitate and the alcohol

[18] Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, 1931, **53**, 350.

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TABLE I.

Supplements	Amt. in gm. per day	No. animals	Average gain in gm. per week over a 5 weeks' experimental period: Short depletion (21-28 days)	Long depletion (56 days)
a. Untreated milk powder	0.3	4	4.1 ± 0.5	
b. Alcoholic extract	0.03	5	4.3 ± 0.3	
c. Alcohol extracted milk powder	0.3	7	2.0 ± 0.5	
d. Ether precipitate	0.07	7	4.2 ± 0.2	
e. Alcohol extracted milk powder	0.3	4		—0.1 ± 0.4 (1 dead)
f. Ether precipitate	0.07	4		6.4 ± 0.3
g. Ether precipitate plus Alcohol extracted milk powder	0.035 + 0.15	4		5.7 ± 0.4
h. Negative controls		17	—1.1 ± 0.3	
		3		1 animal survived experimental period

extracted milk powder by the method[14, 20] of feeding each material alone, and in parallel a combination of ½ the amounts each of ether precipitate and of alcohol extracted milk powder as was fed alone, were not productive of results which could explain the loss of potency in the ether precipitate.

It was, however, noticed that animals continued beyond the usual 5-week experimental period often showed a sharp decline in body weight and this especially when the source of vitamin G was the alcohol extracted milk powder; suggesting that at this time some further growth essential had become necessary through depletion of the bodily stores of the animal of that essential. A series of supplementation experiments were conducted using animals that had been carried through a long depletion period of 56 days (instead of the usual 21-28 days) on the vitamin G deficient diet alone. Table I (e, f, g) gives the results of this work. The same amount of alcohol extracted milk powder as a vitamin G supplement which produced fair growth after 4 weeks' depletion was practically inactive in producing growth after 8 weeks' depletion, Table I(e). However, this extracted milk powder fed in combination with the ether precipitate enhanced the growth-producing power of the latter; thus the combination produced the same growth as that resulting from feeding twice as much of the ether precipitate alone as a source of vitamin G, Table I(f, g), Figure 1.

In the present state of our knowledge, the only satisfactory explanation of this supplementary relation between the 2 fractions would seem to be that a long depletion period (56 days) results in depleting the bodily reserves of the rats of a second limiting factor.

[20] Bisbey, B., Dissertation, Columbia University, 1930.

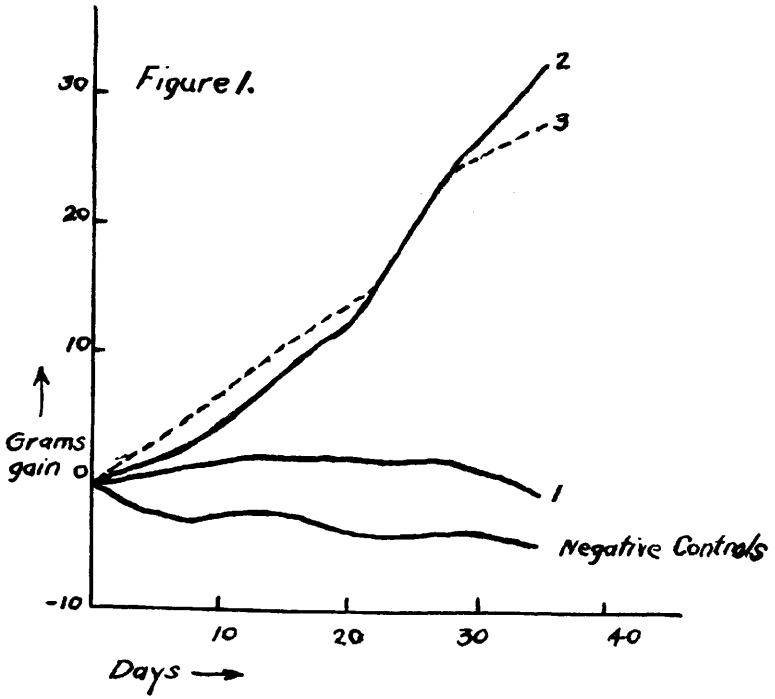

FIG. 1.

Comparison of growth of rats receiving as supplement to a vitamin G deficient diet:

1—0.3 gm. daily of alcohol extracted milk powder.
2—0.07 gm. daily of "ether precipitate."
3—0.15 gm. daily of alcohol extracted milk powder plus 0.035 gm. daily of "ether precipitate."

after 56 days' depletion on a vitamin G deficient diet.

The first limiting factor is carried by both the ether precipitate and the alcohol extracted milk powder, the second factor by the ether precipitate only. This interpretation is applied only as a suggestive hypothesis which best fits the experimental facts so far available.

Summary. 1. A ten-fold concentration of the vitamin G of dried skimmed milk powder has been effected by extraction of the milk powder with hot 93-94% (by weight) ethyl alcohol. Half of the vitamin G activity remained intact in the extracted residue and half was accounted for in the alcohol extract. 2. Evidence for some variant in vitamin G testing has been obtained. This variant may be interpreted as a second limiting substance in vitamin G testing which is preferentially precipitated by addition of ethyl ether to the alcoholic extract of skimmed milk powder. 3. The practicability of the anti-coprophagy harness for increasing the precision of vitamin

G testing is verified by its successful use throughout this investigation.

7318 C

Tuberculosis Induced in the Tadpole by Feeding.*

JOSÉ F. NONIDEZ AND MORTON C. KAHN.

From the Departments of Anatomy and Public Health, Cornell University Medical College, New York City.

Aronson[1] isolated a strain of tubercle bacillus from an iguana which was found dead in the Philadelphia Zoo. A number of tubercles were seen in the lung of this animal and also in the liver. The spleen contained no gross tubercles. A single tubercle was found in the upper pole of the left kidney. In guinea pigs injected intracutaneously with this culture Aronson found that a small ulcer occurred at the site of inoculation. The glands became enlarged and succulent but went on to healing. The organism was found to be pathogenic for the chameleon and salamander and also for the frog. It was not pathogenic for the snake. As regards the frog Aronson's findings have been confirmed by Holly[2] and by the writers with a transfer of this organism which Aronson sent to us sometime ago for further study.

Examination of stained slide preparations reveals the organism to be acid- and alcohol-fast. Ziehl-Neelsen stained vertical sections from growing colonies made with a technique reported by us[3] show some non-acid-fast rods in addition, but the tiny non-acid-fast rods and granules so conspicuous in vertical sections of human and bovine tubercle bacillus colonies are entirely lacking. It would seem then that this organism multiplies principally by simple fission. According to Henderson and Aronson[4] the organism is culturally and serologically identical with *Mycobacterium marinum* which was isolated from salt water fish.

* This study was made in cooperation with the Research Board, National Tuberculosis Association.

1 Personal Communication, Dr. Joseph Aronson, Phipps Institute, Philadelphia, Pa.

2 Personal Communication, Miss Pearl Holly, New York City Department of Health.

3 Kahn and Nonidez, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 577.

4 Personal Communication.