stood quietly a minute or two, a teaspoonful of boiling water, heated over a flame of alcohol held in the lid of a small tin-can, like that of a small-sized George Washington coffee tin.

A useful adjunct in regions where sunshine is rare or not dependable is an electric light bulb, fifteen feet of flexible wire cord, and a two-way plug to screw into the hotel lighting fixture. This will enable the collector to dry out his material while he sleeps.

Needless to say much débris falls into the vial with the arthropods. It is better to sort out the specimens while they are still alive by sprinkling the contents of the vial on a white card and searching it under a small portable dissecting binocular microscope, but if this can not be done at the time, the sorting can be done years later—preserving the specimens with the débris, after the boiling water has cooled, in seventy per cent. alcohol.

ARTHUR PAUL JACOT

MONROE, CONNECTICUT

SPECIAL ARTICLES

THE ADMINISTRATION OF FERROUS IODIDE AND LINOLEIC ACID TO RATS DEPRIVED OF VITAMIN A[1]

MINUTE doses of ferrous iodide were administered daily by Chidester, Eaton and Thompson[2] to rats depleted of vitamin A, and in some cases of vitamin D as well, with the result that for a period of 14 weeks the animals gained in weight at almost the same rate as the positive controls, and were apparently cured of xerophthalmia, although a standard vitamin A-free diet was fed. Chidester, Eaton and Speicher[3] reported approximately the same findings in slightly greater detail. Chidester[4] and Chidester and Wesson[5] discussed the importance of the combinations of dietary substances with respect to iodine, iron and particularly to the unsaturated fatty acids which had just been shown by Burr and Burr[6] to be essential to life. The belief that benefits might accrue to rats deficient in vitamin A from the administration of iodine and of iron, together with essential fatty acids, was put to the test by Chidester, Eaton and Speicher,[7] who fed ferrous iodide and linoleic acid to rats deficient in vitamin A. Resumption of growth and cure of xerophthalmia were reported and interpreted to indicate mobilization of the vitamin stores in the body as well as an actual substitution for vitamin A by the ferrous iodide and linoleic acid.

Neither Mason[8] nor Cameron[9] has been able to confirm the results that Chidester and his associates secured by feeding ferrous iodide to animals depleted of vitamin A. We have likewise been unable to detect any beneficial effects as a result of the administration of ferrous iodide, linoleic acid or of ferrous iodide in combination with linoleic acid.

ANIMAL EXPERIMENTS

Male rats, weighing 38 to 50 gm at 21 days of age, were given *ad libitum* Sherman's diet No. 380. This consists of extracted casein 70 per cent., starch 20 per cent., yeast 5 per cent., salt mixture[10] 4 per cent., and sodium chloride 1 per cent.; in addition, a daily supplement of 0.001 mg of irradiated ergosterol dissolved in oil was administered. When the animals had ceased to grow and had developed severe xerophthalmia (ca. 40 days) they were divided into five groups which received, respectively, daily supplements of 0.1 cc distilled water, 1 drop of cod-liver oil + 0.1 cc distilled water, 0.1 cc ferrous iodide solution (0.1 cc contained 0.024 mg of ferrous iodide), 0.1 cc of linoleic acid, and 0.1 cc of ferrous iodide solution + 0.1 cc of linoleic acid. The supplements, with the exception of the cod-liver oil, were delivered into the mouth by syringe. The water was given to the positive and negative controls to insure the same amount of handling for all the animals. The ferrous iodide solution was made up daily just before use.

All rats were examined at death for infections of the middle ears, of the glands at the base of the tongue and of the salivary glands, as well as for renal and bladder calculi.

RESULTS

The results in Table 1 indicate the complete ineffectiveness of any of the supplements, except cod-liver oil, either for the promotion of growth or the cure of xerophthalmia. At autopsy one or more of the typical

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] F. E. Chidester A. G. Eaton and G. P. Thompson, SCIENCE, 68, 432. 1928.

[3] F. E. Chidester, A. G. Eaton and N. K. Speicher, *Proc. Soc. Exptl. Biol. Med.*, 28, 187. 1930.

[4] F. E. Chidester, *Collecting Net*, 5, 36. 1930.

[5] F. E. Chidester and L. G. Wesson, *Medical Times*, New York, 58, 319. 1930.

[6] G. O. Burr and M. M. Burr, *Jour. Biol. Chem.*, 86, 587. 1930.

[7] F. E. Chidester, A. G. Eaton and N. K. Speicher, *Anat. Rec.*, 47, 304. 1930.

[8] K. E. Mason, *Anat. Rec.*, 51, Supplement 1, Abstract 91. 1931.

[9] H. C. Cameron, SCIENCE, 76, 18. 1932.

[10] T. B. Osborne and L. B. Mendel, *Jour. Biol. Chem.*, 37, 572. 1919.

lesions, associated with vitamin A deficiency, was found in all the rats that did not receive cod-liver oil.

TABLE 1

Rat	Supplement	Duration of depletion	Duration of supplementation	Body weight: Maximum before supplementation	Body weight: Final	Body weight: Change
		days	*days*	*gm*	*gm*	*gm*
C4454	0.1 cc ferrous iodide solution	35	24	108	74	−34
C4478	"	38	29	150	87	−63
C4500	"	38	19	148	113	−35
C4508	"	39	25	143	92	−51
C4612	"	46	16	149	102	−47
Average		39	23	140	94	−46
C4451	0.1 cc linoleic acid	35	40	111	84	−27
C4477	"	38	17	135	90	−45
C4499	"	39	13	119	78	−41
C4506	"	38	11	142	126	−16
C4611	"	31	15	128	87	−41
Average		36	19	127	93	−34
C4450	0.1 cc ferrous iodide solution + 0.1 cc linoleic acid	34	44	113	80	−33
C4476	"	60	8	154	95	−59
C4496	"	38	7	119	106	−13
C4504	"	37	25	80	58	−22
C4610	"	43	10	140	84	−56
Average		42	19	121	85	−36
C4452	0.1 cc water	34	17	106	95	−11
C4502	"	38	12	131	105	−26
C4510	"	45	20	142	82	−60
C4614	"	37	14	102	73	−29
C4615	"	33	46	107	72	−35
Average		37	22	118	85	−33
C4457	0.1 cc water + 1 drop cod-liver oil	39	28 (eyes cured 7 days)	119	200	+81
C4495	"	36	28 (eyes cured 6 days)	100	198	+98
C4613	"	36	28 (eyes cured 6 days)	115	197	+82
Average		37		111	198	+87

The condition of the eyes of the rats that received ferrous iodide alone appeared, upon superficial examination, to be improved, inasmuch as the bloodiness disappeared and the eyes were open. Several rats given supplements of distilled water alone likewise showed this apparent improvement shortly before death. The condition was designated by Dr. A. M. Yudkin, associate clinical professor of ophthalmology at Yale University, as an advanced stage of the xerophthalmia in which the eyes are dry and exhibit Bitot's plaques under the lids. It is possible that Chidester may have mistaken this condition for a cure of the xerophthalmia.

A careful survey of the data presented by Chidester and coworkers led us to the belief that their experimental conditions were not in accordance with the accepted standards of modern vitamin testing. Their animals were depleted for 80 days before supplements were administered. We regard the necessity for so long a period of depletion as an indication either that the diet was not adequately purified from vitamin A, or that the animals had been allowed to store an excessive quantity of this vitamin prior to the experimental period. In either case, at the time supplementation of the diet was begun their animals were 110 days old. Our rats showed marked evidence of depletion of vitamin A in approximately 40 days, and none survived for 80 days unless given cod-liver oil. The control rats in Chidester's experiments were badly stunted, inasmuch as their average weight was only 135 gm at 161 days of age; this was 63 gm less than our control rats that were only 86 days old, and 1 gm less than his best group of experimental rats deprived of vitamin A. That animals, supposedly deprived of vitamin A, should survive for 161 days (131 days on experiment) suggests that an undiscovered source of vitamin A was available. Perhaps some of the confusion has arisen from the fact that a number of the animals employed in Chidester's experiments were depleted of both vitamins A and D, later being given a source of vitamin D. In any event some or all of the possible objectionable factors mentioned—vitamin A in the diet in small amounts, large stores of vitamin A in the rats at the beginning of the experiment, and simultaneous depletion of the animals of vitamins A and D—seem to have resulted in a stunted non-reactive animal which could not be expected to give the clear-cut responses required in vitamin work.

CONCLUSIONS

The administration of ferrous iodide and linoleic acid, alone and in combination, was ineffective as a cure for xerophthalmia, as a stimulant to growth, or as a preventive of infection or renal disturbance in rats deprived of vitamin A.

LUCILLE L. REED
LAFAYETTE B. MENDEL
HUBERT BRADFORD VICKERY
CONNECTICUT AGRICULTURAL EXPERIMENT STATION AND YALE UNIVERSITY