cavity 4 hours after injection of the saline. This residual fluid was fairly rich in protein. Blood counts and determination of hemoglobin before and after the injection of the saline showed no evidence of altered concentration of the blood elements. There was no change in the hemoglobin or the total red cell count.

The rats receiving saline withstood 2200 mg of histamine per kilo of body weight, an amount equal to twice that which is lethal for the normal rat (1100 mg per kg). The results are given in Table I.

Feeding salt in amounts of 1 to 2 g per day for a period of 3 days prior to the test also raised the resistance of the rat but not to the same degree as the saline injections. Some rats survived 1600 mg of histamine per kilo of body weight.

Summary. An excess of salt for short periods above the apparent requirement in rats in which the depôts of saline solution have been filled enhances the natural resistance of the animal to large amounts of histamine. The intraperitoneal injection of large quantities of saline a few hours prior to the test increased the resistance to twice the normal. Salt feeding for several days prior to the test raised resistance about 30 to 40%.

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Whey as the Substratum in Vitamin B_1 Assays.

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To meet the requirements for the other essential substances of the Vitamin B complex in Vitamin B_1 (thiamine) assays, early workers used autoclaved yeast. Because of the uncertainties involved in obtaining a yeast quite free from the vitamin in question, the use of autoclaved whey and autoclaved liver has been advocated since the thiamine in these apparently is more readily destroyed. For these assays it is customary to place the test animals on the basal ration at weaning, hold them until the body stores of B_1 are depleted, manifested by stationary weight, and subsequently for a given specified period add the material to be tested in amounts sufficient for considerably less than the optimum gain. Although theoretically it should be possible to use a test animal for successive assays, in practice it is customary to make only one assay with a given animal. Under these

conditions, possible discrepancies in the basal ration may be masked, or the results may be modified by enrichments in the test material. Furthermore, when the basal ration is made up on the percentage basis, the amounts taken will vary with the appetite of the animal. Thus, in certain instances, less than the optimum amount of the other constituents of the Vitamin B complex may be taken. Therefore, to make sure that the basal ration is adequate, several successive assays should be made with the same animal, allowing sufficient time between tests for the animal to become depleted of its B_1 store. This would seem to be important, even though a negative control accompany the assays.

To determine the value of certain basal rations for Vitamin B_1 assays, successive tests have been made with animals fed *ad libitum* a basal ration consisting of B_1-free casein 20 g, sucrose 62 g, Crisco 10 g, cod liver oil 2 g, and 4 g Hawk and Oser's salt mixture[1] with iron citrate and copper sulphate. In addition, each animal received daily weighed amounts of those substances: autoclaved yeast, autoclaved liver, and autoclaved whey, used to supply the other components of the Vitamin B complex in these assays. These daily doses were consumed before the basal ration was given. After the weight of the animal had become stationary, Vitamin B_1* was given during a 10-day period. In general, the animals continued to gain for several days thereafter, thus making the assay period conform more nearly to the 14-day period suggested by Coward.[2]

The autoclaved dried whey† was prepared by mixing with distilled water, straining through a fine wire mesh and autoclaving in shallow pans at a depth of ¼ inch for 2 hours at 18 pounds. This was subsequently dried and ground. The yeast, a Northwestern dehydrated product, was mixed in 200 g lots with distilled water, rubbed through a fine mesh strainer, diluted with 300± cc N/10 NaOH to bring to pH 7, and boiled 5 minutes. After standing 24 hours, this was again strained and adjusted to pH 7 with added NaOH. The mixture was then autoclaved 5 hours in ¼ inch layers at 18 pounds, subsequently dried and pulverized. After autoclaving, the pH was between 6.8 and 7. The liver (young beef) was finely ground, mixed with distilled water, autoclaved 20 minutes in ¼ inch layers at 18 pounds, dried at room temperature and ground.

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[1] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

* Betaxin, supplied through the courtesy of the Winthrop Chemical Company, Rensselaer, New York.

[2] Coward, K. H., *The Biological Standardisation of the Vitamins*, William Wood and Co., Baltimore, 1938.

† Kindly supplied by the Western Condensing Co., San Francisco, Cal.

TABLE I.
Influence of Various Supplements on Bio-assay of Thiamine.

No. of Animals	Assay period	Supplements: Autoclaved whey	Supplements: Autoclaved yeast	Supplements: Non-autoclaved whey	Avg wt: Initial	Avg wt: End	Avg wt: Gain	Thiamine: Total	Thiamine: For 1 g gain	Thiamine: Range
		g	g	g	g	g	g	γ	γ	γ
4	I		.5	—	77	94	17	39	2.3—	2.1-2.9
3	II		.5	—	88	104	16	39	2.4	2.4-2.7
1	III		.5	—	76	96	20	41	2.1	
3	I	.5	.3	—	80	101	21	48	2.3	1.3-3.5
3	II	.5	.3	—	96	114	18	28	1.6	1.5-2.0
3	III	.5	.3	—	109	125	16	30	1.9	1.4-2.5
1	IV	.5	.3	—	119	134	15	36	2.4	
1	V	.5	.3	—	124	141	17	53	3.1	
3	I	.5	.2	—	77	94	17	43	2.5	1.8-3.2
3	II	.5	.2	—	92	114	22	43	2.0	1.8-2.3
3	III	.5	.2	—	111	128	17	37	2.2	1.9-2.5
1	IV	.5	.2	—	116	136	20	54	2.7	
2	I			.5	66	92	26	169	6.4	6.3-6.4
4	I	.5		—	58	72	14	80	5.7	4.6-7.7*
4	II	.5		—	70	82	12	224	18.6	10.4-28.0
4	III	.5		—	81	89	8	421	52.6	50.0-123.5
4	IV	.5		—	120	135	15	152	10.1	7.0-18.3†
4	V	.5		—	133	141	8	499	62.4	45.0-110.5
5	I	.8 Autoclaved liver		—	67	74	7	155	22.1	15.0-26.3
2	I	.5		—	82	102	20	47	2.4	2.3-2.4

*Animals were given 0.1 g yeast for each of 6 days previous to test to stimulate appetite. No gain during period.
†During 20 days of depletion, animals were given 8 daily doses of 0.5 g autoclaved yeast.

The thiamine which was dissolved in 0.2% HCl solution which previously had been boiled and cooled was stored in a low temperature refrigerator (8°C). To prevent contamination, the pipette used for measuring was kept when not in use in 95% alcohol. The doses of thiamine, which were given first in the day's program to insure complete consumption, were mixed with a small amount of distilled water and a few grains of sugar.

Results. Successive assays of thiamine with the basal ration and 0.5 g autoclaved yeast are consistent from period to period, averaging 2.3γ for one gram of gain; successive assays with the basal ration and 0.5 g autoclaved whey required an increasingly large amount of thiamine for one gram of gain (Table I). That the increase during the succeeding assays in the thiamine requirement is not related to depleted stores of Vitamin B_1 but to a decrease in some substance necessary for growth which is in low concentration in whey is indicated by the animals receiving 0.5 g autoclaved whey and, in addition, 0.2 g and 0.3 g respectively of autoclaved yeast. The amount of B_1 required for one gram of gain is comparable to that required with 0.5 g autoclaved yeast. The inadequacy of whey is further shown by the animal which was placed at weaning on the basal ration with 0.8 g autoclaved whey daily and in addition 2 cc (20γ) thiamine. During the first 10-day period, the animal gained 12 g; during the second and third 10-day periods, he gained 7.4 and 1.0 g respectively. The omission of the thiamine at the end of the third period and the addition of 0.2 g autoclaved yeast daily resulted in a gain of 11.7 g during the subsequent 10-day period (Table II).

The performance of the 4 animals (Table I) which were subjected to 5 successive assays of B_1 with depletion periods between, again shows the inadequacy of whey. Six days previous to Assay I, all animals which had been holding on the basal ration with 0.5 g autoclaved whey were given 0.1 g dried yeast daily to stimulate their appetites; during this period there was no gain in weight. The Vitamin B_1 requirement for one gram of gain was 5.7γ for the first period and 18.6γ and 52.6γ for the 2 subsequent periods. Previous to Assay IV, the animals were given during 20 days of depletion 8 daily doses of 0.5 g autoclaved yeast. Following this, the vitamin required for one gram of gain decreased. During the subsequent period when only autoclaved whey was given during the depletion period, the thiamine requirement seemingly for one gram of gain increased to a value more nearly comparable to the third assay period. Apparently the animals failed to store enough of the unknown factor present in yeast to carry over to the subsequent assay period. In all

TABLE II.
Successive Assays of Thiamine with Autoclaved Whey—No Depletion Periods.

No. of Animals	Assay period	Supplements		Avg wt			Thiamine	
		Autoclaved whey	Autoclaved yeast	Initial	End	Gain	Total	For 1 g gain
		g	g	g	g	g	γ	γ
1	I	.8	—	79.5	91.5	12.0	210	17.5
1	II	.8	—	91.5	98.9	7.4	240	32.4
1	III	.8	—	98.9	99.9	1.0	320	320.0
1	IV	.8	.2	98.9	110.6	11.7	—	—

probability, no more thiamine is needed for gain under one condition than under another, but in an attempt to produce gain in animals depleted of their stores of the unknown factor, more thiamine was used.

Tests during first assays with autoclaved liver were comparable to those with autoclaved yeast. This unknown essential complex is in high concentration in both yeast and liver. It is not destroyed by autoclaving at pH 7 under 18 pounds pressure. Whey contains little, 0.8 g having only slightly more than 0.5 g.

The nature of the substance or substances which are in low concentration in whey has not been investigated. The retarded growth with no other obvious symptoms in animals fed the basal whey ration with repeated doses of Vitamin B_1 during a 3-month test period suggests that one component of Factor W (Frost and Elvehjem),[3] which seems identical with the filtrate factor of Edgar and Macrae,[4] and Edgar, El Sadr, and Macrae,[5] may be the substance in question. In line with this are the findings of Jukes and Richardson[6] to the effect that dried whey is not a concentrated source of the filtrate factor.

Although we have presented no data to this end other than growth and lack of growth, during the course of our experiments we have noted that the animals which received the basal whey ration lacking either B_1 or the unknown factor or both, ate little. It was only when the two were included in the ration that there was any appreciable food consumption. Seemingly appetite hitherto related to B_1 is concerned not alone with B_1 but with a combination of B_1 and this unidentified factor.

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[3] Frost, D. L., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.

[4] Edgar, C. E., and Macrae, T. F., *Biochem. J.*, 1937, **31**, 886.

[5] Edgar, C. E., El Sadr, M. M., and Macrae, T. F., *Biochem. J.*, 1938, **32**, 2200.

[6] Jukes, T. H., and Richardson, C. A., *J. Agr. Res.*, 1938, **57**, 603.