in diameter at the baffle disc converges the air stream and passes it through the baffle into the humidifying chamber. A flange (I) one half inch wide soldered to the small end of the cone prevents water from dripping into the opening. The shaft, shaft hanger, coupling, and collars onto which the fan and agitators are soldered are stock parts of the "Driver" home workshop. They may be purchased from Sears, Roebuck & Co. or from the chain store called "Grant's." The shaft should be of brass, but an iron one may be coated with shellac to prevent rusting. The humidifying chamber may be made easily by a tinsmith from galvanized sheet iron.

When the humidifier is operating continuously and temperature is held constant, the relative humidity within the incubator is a function of the depth to which the agitator discs dip into the water of the humidifying chamber. Hence the relative humidity may be varied by raising or lowering the water level. Since the water level in the humidifying chamber is the same as that in the float chamber of the carburetor, the relative humidity within the incubator may be varied by altering the height of the carburetor. From these facts it is obvious that the precision of humidity regulation depends upon the sensitivity of the float and needle valve in maintaining a constant water level. The ratio between unit difference in water level and unit difference in relative humidity in the incubator depends upon the difference between the capacity of the humidifier and the size of the incubator in question. A machine the size of the one illustrated works very satisfactorily in an incubator of 53 cubic feet capacity.

Other methods of varying the humidity output of the apparatus and therefore of varying the relative humidity in the incubator is to alter the number of agitators or to change the size of the outlet through the baffle plates. These methods, however, serve only to alter the capacity of the humidifier. The sensitivity of the float and needle valve in the carburetor remains as the secret to the accuracy of humidity regulation.

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SPECIAL ARTICLES

THE LEAKAGE OF HELIUM THROUGH PYREX GLASS AT ROOM TEMPERATURE, II[1]

SOME years ago (SCIENCE, 68: 516, 1928) Baxter, Starkweather and Ellestad reported evidence of the slow leakage of helium from a sealed pyrex globe containing helium at room temperature. The gas in the (1044 ml.) globe was originally under slightly less than average atmospheric pressure in this locality, *i.e.*, 75 cm. The globe was occasionally compared in weight with a similar sealed globe, containing argon under a pressure of 79 cm., over a period of a year. In the course of the year the helium globe lost in weight to an extent corresponding to a little more than 1 per cent. of the helium.

Date	Excess in wt. of counterpoise over globe g.	Time days	Loss in wt. mg.	Loss in wt. per day mg.
Nov. 11, 1927	8.08873	0		
Nov. 11, 1928	8.09046	366	1.73	0.00474
Feb. 2, 1929	8.09091	449	2.18	0.00486
April 25, 1929	8.09141	531	2.68	0.00505
May 2, 1929	8.09144	538	2.71	0.00505
June 28, 1929	8.09179	595	3.06	0.00515
March 25, 1931	8.09445	1230	5.72	0.00465
May 22, 1931	8.09500	1288	6.27	0.00488
May 23, 1931	8.09491	1289	6.18	0.00479
May 25, 1931	8.09496	1291	6.23	0.00482

[1] Contribution from the T. Jefferson Coolidge Memorial Laboratory of Harvard University.

The weight of the globe has been occasionally determined since that time and the observations show a continuous regular loss corresponding to that previously found.

In the three years and one half since the experiment was started the proportion of helium which apparently has diffused through the glass is nearly 3.5 per cent. (35 ml.). The rate of leakage per day is somewhat irregular although a continuously slower rate is to be expected on account of the diminishing interior pressure.

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INCREASING THE VITAMIN D POTENCY OF COW'S MILK BY THE DAILY FEEDING OF IRRADIATED YEAST OR IRRADIATED ERGOSTEROL[1]

LUCE[2] in 1924 reported that the diet of the cow appeared to be the main factor in determining the

[1] The experiments here described were carried out through the cooperation of the Walker-Gordon Laboratory Company and Columbia University.

[2] E. M. Luce, *Biochem. J.*, 18, 2379, 1924.

antirachitic potency of her milk. Recently Steenbock and coworkers[3] and Wachtel[4] have shown that the vitamin D activity of cow's milk may be increased several times by supplementing milk-producing rations with irradiated yeast, and Krauss and Bethke[5] have obtained similar results by feeding irradiated ergosterol.

We are briefly reporting here the results of an investigation which was started in April, 1930, and in which both irradiated yeast and irradiated ergosterol were fed and compared as sources of vitamin D for dairy cattle.

Twenty-one Holstein-Friesian cows, each producing more than forty-five pounds of milk daily on a three-time milking schedule, were selected at random for experimentation from among the fresh cows of a large "Certified" dairy herd. They were segregated into seven groups, each of which contained three cows, and produced about the same average quantity of milk. Throughout the experiment, which included a preliminary feeding period of three months and a supplemental feeding period of four weeks, all cows received the same basal ration of alfalfa meal, corn silage, beet pulp and grain mixture. At no time during the experiment did Group I, the negative control group, receive anything except the basal ration and water. During the supplemental feeding period the basal ration of the other six groups was fortified with vitamin D obtained from irradiated yeast and irradiated ergosterol. All cows were confined indoors during the entire experiment except when exercised, to exclude any possible influence of the sun's rays indirectly affecting the antirachitic activity of their milk. They were exercised out-of-doors at midnight in barren corrals.

Throughout the supplemental feeding period each cow in Groups II, III and IV received 10,000, 30,000 and 60,000 rat units per day respectively of vitamin D as irradiated yeast, while each of those in Groups VIII, IX and X received 15,000, 45,000 and 135,000 rat units per day respectively of vitamin D as irradiated ergosterol dissolved in relatively small quantities of corn oil. At the end of the supplemental feeding period equal quantities of milk were collected from each cow during three consecutive days, those from each group being pooled. The butter fats were separated from each composite group sample of milk and carefully filtered at a low temperature to free them from curd, water and salts. These butter fats were then tested for their vitamin D potencies, according to the method described by Steenbock and Black.[6] Several graded levels of each of the butter fats were fed during a ten-day period to rats which had been made rachitic on the Steenbock diet No. 2965. Line tests were made on the radii, ulnae and tibiae of all the animals and the relative values of the different butter fats judged. The vitamin D potencies of the butter fats from the cows fed 10,000, 30,000 and 60,000 rat units of vitamin D in the form of irradiated yeast were approximately 2, 8 and 16 times respectively that of the butter fat obtained from the negative control group, while the vitamin D values of the butter fats from the cows fed 15,000, 45,000 and 135,000 rat units of vitamin D in the form of irradiated ergosterol were approximately 2, 4 and 16 times respectively that of the negative control butter fat. In another experiment to be reported later we have obtained essentially a confirmation of these observations.

While we have demonstrated that diet may very markedly increase the quantity of vitamin D normally occurring in cow's milk, it is equally apparent that some antirachitic supplements may be utilized more efficiently than others by the dairy cow. Steenbock and coworkers[3] state that they have evidence to show that the vitamin D in yeast occurs in at least two different forms. This may possibly account for the different effects produced by irradiated yeast, and irradiated ergosterol. On the other hand, inasmuch as Hart and coworkers[7] demonstrated that eight ounces of cod liver oil daily were poorly absorbed by milking cows it might be argued that in our experiments the ergosterol dissolved in corn oil likewise was poorly absorbed. It should be pointed out, however, that in contrast to the large amount of oil fed by the Wisconsin investigators the largest amount received by any of our cows per day was 90 cubic centimeters, equally distributed in three gelatine capsules, one being fed every eight hours. Furthermore, when compared with each cow's ration of 30 pounds silage, 12 pounds of grain concentrate, 12 pounds of alfalfa hay and 4 pounds of beet pulp, it would hardly be expected that this small volume of oil would interfere with the absorption of vitamin D from the irradiated ergosterol dissolved in it.

The fact that the vitamin D potency of cow's milk can be increased at least 16 times by feeding is important; for in this way milk, with its high content and excellent proportions of calcium and phosphorus, can be made a still better food for the development of the skeletal system.

[3] H. Steenbock, E. B. Hart, F. Hanning and G. C. Humphrey, *J. Biol. Chem.*, 88, 197, 1930.

[4] M. Wachtel, *Münch. Med. Wochschr.*, 76, 1513, 1929.

[5] W. E. Krauss and R. M. Bethke, April, 1931, Meeting, Am. Soc. Biol. Chemists.

[6] H. Steenbock and A. Black, *J. Biol. Chem.*, 64, 263, 1925.

[7] E. B. Hart, H. Steenbock and E. C. Teut, *J. Biol. Chem.*, 84, 359, 1929.

Full reports of these investigations which are being continued will be published later.

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THE PREPARATION OF ADRENAL EXTRACT

SINCE we have received many requests for the details of our method of preparing an extract of the adrenal cortex, their publication seems desirable.

The medulla contains toxic substances[1] which must be removed if whole adrenals are to be used. We have tried to remove or destroy these substances by washing the ethereal solution with acid or alkali, or by treating the alcoholic or aqueous solutions with aluminum hydroxide, Lloyd's reagent, permutit, kaolin or charcoal. The toxic substances can be removed, but so much cortin is also removed that at present it seems more satisfactory to start with cortex alone.

Fresh adrenal cortex, or cortex from adrenals frozen immediately after killing, is finely ground in a meat chopper. Peroxide-free ethyl ether (a one per cent. $K_2Cr_2O_7$ solution acidulated with H_2SO_4 turns blue in a few seconds after shaking with peroxide ether) is added to the material in a flask as soon as possible. Ether must always be kept in the dark, because peroxides develop within a few hours upon exposure to light. We, therefore, work in a dark room or cover our flasks with opaque cloth. Peroxides destroy cortin and cause toxic substances to develop. A satisfactory proportion for extraction is 4 liters of ether to 3 kilograms of tissue in a 12 liter flask. The air in the flask is replaced with CO_2 and the flask closed with a rubber stopper wired in place. Extraction is facilitated by agitation on a shaker or rocker for four to eight hours. Care must be taken not to produce an emulsion by too vigorous shaking. After pouring off the ether extract, second and third extractions with ether are made in a like manner. The three ether extracts are combined and concentrated almost to dryness by vacuum distillation.

The residue from the ether distillation is extracted four times with 95–98 per cent. ethyl alcohol heated from 45° to 50° C. and kept warm during the extraction so that the fatty material is kept fluid, otherwise the alcohol does not penetrate. For one kilo of gland material about 50–60 cc of alcohol are used in each extraction. One hour on the shaker is adequate for each extraction. The flask is cooled by surrounding with cracked ice so that the alcohol may be easily separated from the oily matter. All fractions are combined and enough water added to make the alcohol content 80 per cent. The solution is chilled to −10° C. ($CaCl_2$ and ice mixture) or below and filtered in a cold atmosphere (4° C. or less) to remove undesirable material. This is extremely important. If the chilling is not sufficient or the solution becomes warmed during filtering, toxic substances are carried through the paper. The alcohol is removed *in vacuo* and the residue again extracted with a small volume of 60–75 per cent. alcohol, then chilled and filtered as above. The alcohol is again removed and the residue extracted with a small volume of ether. The ether is driven off and its residue taken up with sufficient water to make the desired concentration. NaCl is added to make the extract isotonic with the body fluids. After passing through a Seitz filter, the extract is ready for injection.

If the precautions are carefully observed a potent, non-toxic extract can be made. One patient was injected four times daily for more than seven months without untoward effects.

Recently, Britton and Silvette[2] compared our method with that of Swingle and Pfiffner. One of the investigators from Britton's laboratory spent several weeks working with Swingle and Pfiffner,[3] while their knowledge of our method was based only upon the brief published[4] outline of our process. The results of Britton and Silvette are, therefore, what one might expect. Perla and Marmorston-Gottesman[5] have recently used our method successfully. Dr. Perla spent two days in our laboratory.

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THE LIFE HISTORY OF BABESIA BIGEMINA IN THE NORTH AMERICAN FEVER TICK

Babesia bigemina (Smith and Kilbourne, 1893)[1] is the piroplasm which is the causative agent of Texas cattle fever, a disease that at one time threatened the existence of a cattle industry in the United States. Smith and Kilbourne (1893) demonstrated conclu-

[1] E. B. McKinley and N. F. Fisher, *Am. J. Physiol.*, 76: 268, 1926.

[2] S. W. Britton and H. Silvette, SCIENCE, 73: 322, 1931.

[3] W. W. Swingle and J. J. Pfiffner, *Am. J. Physiol.*, 96: 153, 1931.

[4] F. A. Hartman, K. A. Brownell and W. E. Hartman, *Am. J. Physiol.*, 95: 670, 1930.

[5] D. Perla and J. Marmorston-Gottesman, *Proc. Soc. Exp. Biol. and Med.*, 28: 650, 1931.

[1] T. Smith and F. Kilbourne, *Bull.* Bur. Animal Industries, U. S. Dept. Agr., Washington, 1: 177–304, 1893.