

4,000 rabbits have been collected from the area and have been given minute examination. As the rabbits during the summer time have averaged over 2,000 ticks, there has been ample opportunity to observe the spread of this virus if it were present in the ticks. Many experiments involving the feeding of ticks upon rabbits have been carried out with the collections from the Lake Alexander Area without production of rabbit horns. The possibility of a spontaneous development of the tumors described above would therefore appear to be excluded by the origin of both the experimental animals and the ticks from the Lake Alexander Area.

The development of the experimental horns only on the one ear infested with ticks and the appearance of numerous foci of development within a two-day period and at a time corresponding to the incubation period of the infection, point directly to the tick bites as the source of infection.

Our successful transmission of rabbit horns by the feeding of ticks shows only a mechanical transfer, but demonstrates that sufficient virus may be carried by an insect bite to produce successful inoculation. It appears probable that this infection may be likewise transferred by mosquitoes and biting flies.

8442 C

The Vitamin-B and -G Content of Commercial Beer.

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Since beer is being advertised by several commercial companies as a source of vitamins, it is desirable to have quantitative information on its vitamin content. The only published studies on this question were made on European beers more than 8 years ago. Scheunert and Schieblich¹ and also Southgate² demonstrated vitamins of the B complex in beer and reported further that A and C are very low or absent. On the other hand, Harden and Zilva,³ while agreeing on the absence of C, were unable to find B in the beer which they examined.

¹ Scheunert, A., and Schieblich, M., *Chemie der Zelle und Gewebe*, 1924, **12**, 45; *Biochem. Z.*, 1927, **186**, 229.

² Southgate, H. W., *Biochem. J.*, 1924, **18**, 769.

³ Harden, A., and Zilva, S., *Biochem. J.*, 1924, **18**, 1129.

In the present study, the B-complex concentration of several commercial beers was determined by the rat-growth method. Other points studied were a comparison of pasteurized and unpasteurized beer, the effect of filtration through paper in removing vitamins, and the relative concentrations of B and G.

Experimental methods. Healthy albino rats, weighing 35 to 45 gm. were weaned when 21 days old and depleted of vitamins of the B complex on the following basal diet:

Purified casein	20%	Agar-agar	5%
Sucrose	12	Hydrogenated cotton seed oil	12
White dextrin	45	Cod liver oil (standardized for vita-	
Complete inorganic salt mixture....	4	mins A and D)	2
and distilled water <i>ad lib.</i>			

A depletion period of 2 weeks on this diet sufficed to stop all growth in rats weaned at 21 days, so that they could be considered free from stored vitamins. The depleted rats were then started on a test period during which one rat of each litter was continued on the basal diet while its littermates were fed the basal diet plus measured daily doses of the test materials. All of the rats had free access to the basal diet and distilled water, except that water was withheld in those cases in which it might restrict the consumption of beer, because of the water content of the beer.

The rats were confined in galvanized wire cages having screen bottoms—2 meshes to the inch—during both the depletion and the test periods. Littermates were confined together during the depletion period, but they were separated into groups—according to litter, sex, and weight—and each rat was isolated from his fellows during the test period. The daily doses of the test materials were fed separately each morning and the amount eaten each day was carefully noted. The rats were inspected daily, especially for symptoms of B or G deficiency, and their weights, taken before the morning feeding, were recorded for each week of the test period.

Five different beers, A, B, C, D, and E, were fed as supplements to the basal diet. Three of these beers, A, B, and C, came from a local brewery, whereas the others, D and E, came from breweries in 2 other cities. The beers C, D, and E are pasteurized beers which were purchased from retailers in the ordinary 12-ounce bottles, and the beers A, C, and D are advertised as a source of vitamins. The beers A and C are the same except that C is pasteurized. The local beers, A, B, and C, were fed at various dose levels from 2.5 cc. to 30 cc. per rat per day, but the other beers, D and E, were fed only at the 10-cc. level.

It was our experience that weanling rats will not consistently drink more than 10 cc. of beer per rat per day, even when they are denied water. In order to feed more than 10 cc., we evaporated the beer at 38°C. either to dryness or to a thin syrup (2/15 the original volume) and fed weighed or measured quantities of the concentrate. The rats would readily consume either the solids or syrup equivalent to 30 cc. of the original beer, but they would not consistently eat more than this amount. Comparisons of the evaporated and unevaporated beers at the same dose-level have demonstrated that no appreciable loss of vitamins of the B complex takes place during the evaporation at 38°C.

TABLE I.
Growth of Rats with Different Beer Supplements.

Supplement	No. of rats	Weaning weight	Depl'n weight	After 4 weeks		After 8 weeks	
				No. alive	Weekly gain	No. alive	Weekly gain
0.4 gm. dried yeast	8	gm. 35	gm. 37	8	gm. 22.3	8	gm. 19.6
No supplement	8	39	40	0	—	—	—
30 cc. A	8	41	44	8	12.7	8	11.5
30 " B	8	40	40	8	11.0	Not tested	
30 " C	8	38	39	8	11.5	"	"
10 " A	8	37	41	8	2.8	1	—
10 " B	4	42	45	2	2.2	0	—
10 " C	4	41	42	2	3.5	1	—
10 " D	4	39	41	4	3.0	1	—
10 " E	4	40	42	2	1.5	0	—

Results. Table I shows the rat growth and survival when no supplement was given, and when 0.4 gm. dried yeast, or the 5 beers at 2 dose-levels were used as supplements. The figures show that with 30 cc. of the beers A, B, and C, the growth is about one-half that obtained with 0.4 gm. dried yeast; and further that there is no significant difference among these 3 beers at this dose level. At the 10-cc. level the beers A, C, and D, which are advertised to contain vitamins, show about 50% more growth than beers B and E, which are not so advertised. This difference is hardly significant in view of the small number of animals which survived more than 4 weeks at this dose-level; the point to be emphasized, we feel, is that there is not more than 50% difference. The pasteurized beer C allows the same growth as the unpasteurized sample A, from which it was prepared, showing that pasteurization does not change the vitamin-B content of beer. At the 2.5- and 7.5-cc. levels, the rats showed slow (1 or 2 gm. per week) and erratic growth in the first 2 weeks, and then rapidly lost weight and died. At the 15-cc. dose level, there was an average weekly gain of about 5 gm., with no significant difference between the various beers.

None of these rats showed any symptoms of G deficiency. At the 30-cc. dose-levels, no symptoms of B deficiency were shown in 8 weeks, and at the 15-cc. level, none appeared in 4 weeks. At the 10-cc. level, polyneuritis (vitamin B deficiency) was observed in all rats which died between the fifth and eighth weeks.

All of the beers fed in this experiment were filtered at the brewery, and were clear and free from suspended yeast particles. In some other studies, unfiltered beer obtained from the brewery, was filtered at the laboratory, either through sintered glass or through filter paper. The sintered glass filtrates showed no appreciable difference in vitamin content from the brewery filtered beer; whereas filtration through paper caused a loss of some B and nearly all of the G from the beer. In one experiment, only 6 of 12 rats receiving 15 cc. of paper-filtered beer survived a 4-weeks' test period; and although these 6 rats showed an average weekly gain of 3.7 gm., they had practically stopped growing at the end of 4 weeks, and all showed the sunken eyes, bald eyelids, bloody noses, and extensive loss of hair characteristic of G deficiency. These symptoms were never observed among the rats receiving beer filtered at the brewery, or through sintered glass. Incidentally, this observation may explain the failure of Harden and Zilva to find evidences of vitamin-B complex in the beer they investigated; for they mention that they took precautions(?) to see that the beer they used was clear and free from suspended yeast particles.

Table II shows the results of an experiment in which a B extract (containing minimal amounts of G) and autoclaved yeast (G with minimal amounts of B) were fed together, separately, and separately as additions to beer supplements. The B extract and autoclaved yeast were prepared according to Guerrant and Dutcher.⁴ That these supplements were satisfactory for the experiment is shown by the results with the first 3 diets: with both B extract and autoclaved yeast, very good growth, 15 gm. per rat per week, was obtained; with the B extract alone, poor growth (3 gm. per week) and definite symptoms of G deficiency after 4 weeks; with the autoclaved yeast, there was fair growth in the first 4 weeks, 7.8 gm. per rat per week, with all rats dying during the second 4 weeks showing polyneuritis.

When these supplements are added to 30 cc. of beer A, it is seen that while the addition of the B extract increases the growth (15.7 gm. per rat per week) to that observed in the positive control, the addition of the autoclaved yeast increases it to only 12.5 gm. This

⁴ Guerrant, N. B., and Dutcher, R. A., *J. Biol. Chem.*, 1932, **98**, 225.

TABLE II.

Supplement	No. of rats	Weaning weight	Depl'n weight	After 4 weeks		After 8 weeks	
				No. alive	Weekly gain	No. alive	Weekly gain
per rat per day		gm.	gm.		gm.		gm.
0.4 gm. autoclaved yeast + B extract equivalent to 0.6 gm. yeast	3	39	37	3	15	3	15.9
B extract	3	47	51	3	3.5	3	2.7
Autoclaved yeast	4	41	48	4	7.8	0	—
30 cc. Beer	4	45	50	4	11.5	4	10.1
30 cc. Beer + B extract	4	42	49	4	15.8	4	15.6
30 cc. Beer + autoclaved yeast	4	43	46	4	12.5	4	12.0

indicates that the limiting factor in the growth of the rats with beer supplements is B rather than G, and that beer contains relatively more G than B. This conclusion is supported by the observation previously mentioned, that we never observed symptoms of G deficiency in animals receiving beer supplement (except the paper-filtered beer); although we consistently observed polyneuritis with beer supplements of 10 cc. or less, after 4 weeks on this diet.

These data permit a calculation of the approximate concentration of vitamins B and G of the beer in standard units. Sherman⁵ defines a unit of vitamin B and vitamin G as that amount which, when fed daily under prescribed experimental conditions, induces a growth in standardized rats at a rate averaging 3 gm. per week over a test period of 4 to 8 weeks. Since 30 cc. of beer caused a growth of about 12 gm., and 10 cc. of about 3 gm. per rat per week, one calculates about 0.1 Sherman unit per cc. of beer or about 3 Sherman units per ounce. The least effective beer, E, shows a concentration of about one-half of this. Since the limiting factor in the growth of the rats is B rather than G, these concentrations refer to vitamin B. The concentration of G is considerably greater, probably at least twice that of B. Since beer contains about 7% by weight of solids, it appears that the beer solids contain about one-tenth as much vitamin B as does dried yeast. Expressed in terms of ordinary commercial yeast, a one-half ounce yeast cake is equivalent to 25 ounces of beer in its vitamin-B content. The vitamin-B concentration which we found agrees approximately with that found by Scheunert and Schieblich¹ in their bottom fermented (Pilsner) beers, but is distinctly less than that of their top fer-

⁵ Sherman, H. C., *Chemistry of Food and Nutrition*, (4th edition), New York, 1932, 389 and 405.

mented (Porter) beer, for which they report that 5 liters of beer for 60 kg. rat promote a growth which is not entirely normal. Our results show the vitamin-B concentration of beer to be approximately the same as that of milk.⁶

These results show that beer is a wholly inadequate source of vitamin B for the rat. When allowed free access to beer, this animal will not drink enough (at 10 cc. per day) to prevent marked symptoms of polyneuritis; if allowed free access to beer solids or concentrates, it will eat only enough (at the equivalent of 30 cc. of beer per day) of these to show about one-half normal growth because of B deficiency. Assuming that the vitamin-B requirement of the human species per unit weight is not less than one-third that of the rat, a 30-pound child eating the equivalent of 1 liter of beer per day, would show marked symptoms of B deficiency, unless this vitamin were supplied from another source; and even at 3 liters of beer per day there would be subnormal growth.

Summary. By the rat growth method, 5 samples of commercial beer, 3 of which were advertised as a source of vitamins, were found to contain from 0.05 to 0.1 Sherman units of vitamin B per cc. of beer. The concentration of vitamin G is greater than that of B. Pasteurization does not change the concentration of these vitamins. Filtration through paper removes practically all of the vitamin G from beer; filtration through sintered glass has no such effect.

At best, beer is a very poor and inadequate source of vitamin B for rats, and probably also for humans.

8443 P

Adrenal Glands in Dogs with High Intestinal Obstruction.

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In high intestinal obstruction in man,¹ in the dog,² and in the monkey³ the essential changes in the blood chemistry are primarily

⁶ Macy, I. G., and Outhouse, J., Graham, A., and Long, M. L., *J. Biol. Chem.*, 1927, **73**, 189; Samuels, L. T., and Koch, F. C., *J. Nutrition*, 1932, **5**, 307.

¹ Haden, R. L., and Orr, T. G., *Bull. Johns Hopkins Hosp.*, 1923, **39**, 26.

² Haden, R. L., and Orr, T. G., *J. Exp. Med.*, 1923, **37**, 365.

³ Haden, R. L., and Orr, T. G., *J. Exp. Med.*, 1925, **41**, 107.