The tumor may occur in any segment of the larva but seems to occur more often in the twelfth and thirteenth segments. When the tumor occurs in the thoracic region, there may be an ingrowth of tumor cells into the imaginal discs of the appendages checking the development of these parts.

The cells of the tumor are rounded or polygonal in shape and show the presence of pigment.

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The suitability of the "Bachman Test" for water-soluble B.

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Two recent publications by Drs. Bachman[1] and Williams[2] dealing with the vitamine requirements of yeasts suggest that the methods developed are adaptable to quantitative measurement of vitamine content (B variety). At the suggestion of the senior author Miss Stevenson has conducted experiments with both methods and in this report are presented some of the results with the Bachman test.

Briefly this method consists in planting yeast cells in a culture medium (Nageli's solution: 100 c.c. distilled water; 10 gms. dextrose; 1 gm. ammonium nitrate; 0.05 gms. calcium phosphate; 0.5 gms. potassium acid phosphate; 0.25 gms. magnesium sulfate) contained in a Durham or Smith fermentation tube and incubating the tubes at 28–32° C. to obtain gas formation. To these tubes are added vitamine "B" extracts from various sources and Dr. Bachman's results showed that in the absence of such extracts gas formation either fails to take place or at least very slowly.

Our experiments aimed to confirm Dr. Bachman's results, to determine whether the method gave promise of use quantitatively and whether it might be used to detect the "B" vitamine qualitatively. The results of our experiments follow:

[1] 'The "Vitamine Requirements of Certain Yeasts," by Freda Bachman, *Jour. Biol. Chem.*, XXXIX, 235 (1919).

[2] "A Simple Biological Test For Vitamines," by R. W. Williams, *Journ. Biol. Chem.*, XXXVIII, 465 (1919).

EXPERIMENT 1. To test the applicability of the method to "B" Vitamine extract as prepared by McCollum[1] from Navy Bean.

Contents of Tubes.	Per Cent. of Gas-formation by Days.								
	1.	2.	3.	4.	5.	7.	8.	9.	14.
1. Control tube. Contained Nageli solution and one loopful of yeast cell suspension..	0	0	0	0	0	0	0	0	0
2. Control tube 2. Contained Nageli solution, loopful yeast cell suspension and 1 c.c. of a dextrin solution made by dissolving 20 gms. dextrine in 150 c.c. of distilled water. The dextrin solution was sterilized in the Arnold machine at 100° C.	0	0	0	0	0	0	0	0	0
3. Control tube 3. Contents same as tube 2 but the dextrin solution was sterilized again, after Arnold treatment, for 20 minutes in the autoclave at 15 lbs. and about 120° C.....	0	0	0	0	0	0	0	0	0
4. Tube contained Nageli, loop of yeast and 1 c.c. of a dextrine vitamine extract from Navy Bean (for preparation see explanation below) sterilized in the Arnold at 100° C.....	0	90	87	63	18	upset at this point			
5. Same as tube 4 but sterilized in the autoclave in addition to Arnold sterilization..	0	93	85	58	56	40	38	37	33

Explanation: The vitamine dextrine solutions were obtained by first extracting 86 gms. of Navy bean flour with ether in the Soxhlet for 18 hours. The residue was then extracted for two six hour periods with boiling alcohol (95 per cent.) in a reflux condenser. The alcohol extract was mixed with 20 gms. of dextrine and evaporated to dryness. The solution was made by dissolving this dextrin vitamine in 150 c.c. of distilled water. The control dextrin solution was made by dissolving 20 gms. of dextrine in 150 c.c. distilled water. All the solutions were subjected to two 30 minute periods in the Arnold sterilizer with a 24-hour incubation period intervening. The materials tested in tubes 3 and 5 were then given an additional 20 minutes in the autoclave at 15 lbs. and approximately 120° C. A pure culture of the Fleischman round yeast was used. The suspension was made by transferring to 5 c.c. of sterile water several loopfuls of the agar slant culture and the tubes were inoculated with one loopful of this suspension. Our tubes were incubated at 32° C. The CO_2 is very rapidly reabsorbed by the Nageli solution and sealing the tube with mineral oil was not effective. Consequently readings may be discontinued after the per cent. of gas reaches the maximum.

[1] "A Study of the Dietary Essential, Water-soluble B.," by E. V. McCollum, *Journ. of Biological Chemistry*, XXXIII, 55.

EXPERIMENT 2. To determine the effect of variation in the concentration of the dextrine vitamine solution.

Tube Contents	0.1/Cc. Dextrine V.						0.2/Cc. Dextrine V.					
Sterilization Method	Arnold.			Arnold Plus Autoclave.			Arnold.			Arnold Plus Autoclave.		
Series	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.
Days:												
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	16	2	1	50	0	0
3	0	0	0	0	0	0	63	35	78	70	18	75
4	0	0	0	0	0	0	29	25	99	35	30	67
5	0			0			11			72		
7	0	1	3	0	0	0	10	8	79	62	7	22
8	0			0				5		52		
9	0			0			4			48		
14	0			0			3			45		

Tube Contents	0.5/Cc. Dextrine V.						1.0/Cc. Dextrine V.					
Sterilization Method	Arnold.			Arnold Plus Autoclave.			Arnold.			Arnold Plus Autoclave.		
Series	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.
Days:												
1	0	0	0	0	0	0	0	0	0	0	0	0
2	95	95	53	26	60	23	90	99	68	93	99	68
3	91	100	95	51	100	100	87	100	100	85	100	100
4	85	99	95	58	84	96	63	75	82	58	65	80
5	68			25			18			56		
7	10	10	10	8	12	8	40	20	8	u	30	28
8	8			7			38			u		
9	8			7			37			u		
14	7			6			33			u		

Explanation: The only variants were the amounts of vitamine solution used and the yeast suspensions.

All the Vitamine solutions were sterilized as in experiment 1 and the second set in the Autoclave for additional 20 minutes. In series 2 and 3 a new yeast suspension was made. The Vitamine solution was the same as used in Experiment 1.

EXPERIMENT 3. To determine the suitability of the test as a qualitative agent in determining the presence and relative amount of "B" Vitamine.

N.B. All tubes contained the Nageli solution and a loopful of yeast suspension as in Experiments 1 and 2. In this experiment only Arnold sterilization was used (two 30-minute periods with 24 hours incubation period intervening).

Tube Contents.	Per Cent. of Gas Formed by Days.						
	1.	2.	3.	4.	5.	6.	7.
1. 1 c.c. of a vitamine extract of a commerical farina. 110 gms. farina was used and the extract made up to 150 c.c.	0	60	100	83	33	28	
2. Vitamine extract from 25 gms. alfalfa. Extract made up to 150 c.c. Using 0.5 c.c.	3	98					
Using 1 c.c.	24	97			48		
4. Protozoa food mixture after Calkins made by boiling 130 gms. flour with 100 gms. timothy hay and 100 c.c. distilled water, allowing to cool and decanting.							
Used 0.5 c.c.	0	0	0	0	0	0	0
Using 1.0 c.c.	0	0	15	32	12	18	
5. 35 c.c. of the Protein free milk obtained from 1 qt. milk	0	20	40	53			
6. 1 c.c. Walker Gordon whole milk	0	100	100	100	100	60	
7. Duplicate of (6) with varying concentrations:							
Using 1 c.c.	0	68	100	100	86		
Using 0.5 c.c.	0	52	100	88	50		
Using 0.3 c.c.	0	25	60	50	32		
8. Cow's milk to which was added varying amounts of N(NaOH) solution before sterilizing.							
Using 1 c.c. of solution (25 c.c. milk cont. 1 c.c. N(NaOH))	0	3	70	100			
Using 1 c.c. of solution (25 c.c. milk cont. 0.5 c.c. N(NaOH))	0	4	58	95			
Using 1 c.c. of solution (25 c.c. milk cont. 0.4 c.c. N(NaOH))	0	55	100	100			
Using 1 c.c. of solution (25 c.c. milk cont. 0.3 c.c. N(NaOH))	0	55	100	100			
Using 1 c.c. of solution (25 c.c. milk cont. 0.2 c.c. N(NaOH))	0	60	100	100			
Using 1 c.c. of solution (25 c.c. milk cont. 0.1 c.c. N(NaOH))	0	55	100	100			

9. Breast milk from six different sources. Each specimen used sterilized two 30-minute periods in the Arnold Sterilizer before using. 1 c.c. used in each inoculation. 3 series of experiments. Series 1 used a different yeast suspension from that used for Series 2 and 3.

Sources.	Series 1.							Sources.	Series 2.					Sources.	Series 3.				
	1.	2.	3.	4.	5.	6.	7.		1.	2.	3.	4.	5.		1.	2.	3.	4.	5.
A. 1 c.c. . .	0	12	63	80	100	100	100	1 c.c.	0	0	60	100	68	0.5 c.c.	0	0	0	10	30
B. 1 c.c. . .	0	21	59	70	70	65	65	1 c.c.	0	31	70	72	62	0.5 c.c.	0	0	24	65	90
C. 1 c.c. . .	0	17	47	68	82	68	65	1 c.c.	0	8	82	60	65	0.5 c.c.	0	3	33	50	48
D. 1 c.c. . .	0	21	72	72	72	68	68	1 c.c.	0	65	90	80	70	0.5 c.c.	0	0	28	78	90
E. 1 c.c. . .	0	1	23	56	65	45	44	1 c.c.	0	5	70	90	65	0.5 c.c.	0	0	20	68	64
F. 1 c.c. . .	0	0	20	35	59	55	54	1 c.c.	0	3	78	72	45	0.5 c.c.	0	0	48	68	50

CONCLUSIONS.

The results of the various experiments are obvious from the records. It need merely be pointed out that in none of the duplicates were we able to repeat the gas figures exactly and that for quantitative measurement the test needs further standardization to be efficient in comparing solutions. The great variability of the yeast loopfuls obtained in this method would easily give rise to considerable variation and experiments are being made along this line to be reported later.

As a means of studying presence of "B" Vitamine in large or small quantity and as an index more reliable than rat feeding experiments the test offers such marked advantages in sensitiveness and in speed of observation that it seems well worth while to devote more time to its improvement.

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Two sex-linked lethals of simultaneous appearance in Drosophila obscura.

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A pair mating in *Drosophila obscura* (Fallén) produced a sex ratio of 106 females to 22 males. This was about a 1:4 ratio and indicated a case of two sex-linked lethals, or lethals at two loci on the X-chromosome. Both lethals (l_1 and l_3) appeared simultaneously in the same culture from a female whose mother did not carry a sex-linked lethal, as was shown by the normal sex ratio produced by her; and the father could not have carried such a lethal and lived.

Three daughters inherited both lethals in the same chromosome with a sex-linked gene producing the character "short" wing veins. These three females produced a total of 352 females to 40 normal and 50 "short" males. Such a count suggested that the gene for "short" was between the two lethals, which were far enough away from it for each to segregate almost independently from it,