THE PRODUCTION OF HISTAMINE IN BACTERIAL CULTURES

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In a previous paper (Eggerth, Littwin, and Deutsch, 1939) a simple and convenient method for the quantitative determination of histamine in bacterial cultures has been described. By this method, the cultures are made alkaline with sodium carbonate, then extracted in a special apparatus with a mixture of chloroform and amyl alcohol, the histamine passing into a layer of dilute sulfuric acid. Ammonia, volatile amines, and amyl alcohol are removed from this extract by boiling at pH 9.2. The histamine is determined colorimetrically by the method of Koessler and Hanke (1919a). The presence of histamine is then confirmed qualitatively by forming the di-picrate.

In the present investigation, a series of organisms has been studied to determine which of them are capable of producing histamine, and what factors influence histamine production.

A number of culture media were employed, as the composition of the medium profoundly affects the amount of histamine produced. A detailed report will be made of the following seven only. Histidine is an essential ingredient for all of them, for, with the exception of *Clostridium welchii*, none of the organisms studied were able to form histamine unless free histidine was present.[1]

The first five media were adjusted to pH 7.6 and autoclaved; then enough sterile concentrated glucose solution was added to

[1] Suzuki and Joshimura (1909) have shown that infusions of the flesh of certain sea fish are rich in free histidine. In the present investigation, it was found that many organisms produced high yields of histamine on a medium consisting of fresh mackerel infusion with asparagine or peptone and glucose, with no added histidine.

give a concentration of 1 per cent. Medium 6 was also adjusted to pH 7.6, but 2 per cent of glycerol was added instead of the glucose. The media were then distributed in sterile tubes, 12 cc. per tube.

No. 1. Ammonium-nitrate-histidine-glucose medium:

300 cc. H_2O
0.3 gram histidine di-hydrochloride
0.3 gram $(NH_4)_2SO_4$
0.6 gram $NaNO_3$
0.6 gram $Na_2HPO_4 \cdot 12H_2O$
0.3 gram KCl
0.05 gram $CaCl_2$
0.05 gram $MgSO_4$

Many organisms failed to grow on this medium. A control determination made on the uninoculated medium gave no color with the diazo reagent.

No. 2. Asparagine-histidine-glucose medium:

300 cc. H_2O
1.0 gram asparagine
0.3 gram histidine di-hydrochloride
0.6 gram $Na_2HPO_4 \cdot 12H_2O$
0.3 gram KCl
0.05 gram $CaCl_2$
0.05 gram $MgSO_4$

A control determination on the uninoculated medium gave no color with the diazo reagent.

No. 3. Asparagine-histidine-cysteine-glucose medium. This has the same formula as medium 2, but with the addition of 0.3 gram cysteine hydrochloride.

No. 4. Egg-yolk-asparagine-histidine-glucose medium. Seven parts of distilled water were added to 1 part of fresh egg yolks and stirred. The mixture was heated in the Arnold sterilizer for 1 hour and filtered.

300 cc. egg yolk infusion
0.3 gram histidine di-hydrochloride
1.0 gram asparagine
0.6 gram $Na_2HPO_4 \cdot 12H_2O$
0.3 gram KCl
0.05 gram $MgSO_4$

A control determination on the uninoculated medium gave a color reading of 0.3 mm. per cubic centimeter. This medium gave excellent growth with most organisms, and the yields of histamine were high.

No. 5. Meat-extract-peptone-histidine-glucose medium:

300 cc. H_2O
1.2 grams meat extract
3.0 grams peptone (Parke Davis)
0.3 gram histidine di-hydrochloride

A blank determination on the uninoculated medium gave a color reading of 5.6 mm. per cubic centimeter which matched the histamine standard fairly well.

No. 6. Meat-extract-peptone-histidine-glycerol medium. This medium has the same formula as the preceding one, except that the carbohydrate added is 2 per cent glycerol.

No. 7. Histidine alone, with no other source of nitrogen and no carbohydrate:

300 cc. H_2O
0.3 gram histidine di-hydrochloride
1.2 grams $Na_2HPO_4 \cdot 12H_2O$
0.3 gram KCl
0.05 gram $CaCl_2$
0.05 gram $MgSO_4$

Acetic acid or sodium hydroxide were added to obtain the desired pH. Little or no growth is to be expected in this medium; it was used to determine histamine production by "resting" bacteria. A blank test on the uninoculated medium gave no color with the diazo reagent.

In calculating the amount of histamine produced in a culture, a correction was made in each case for the blank for that medium, in addition to a correction of 0.3 mm. for the color of the reagent.

I. THE RATE OF HISTAMINE PRODUCTION IN BACTERIAL CULTURES

Koessler and Hanke (1919b) determined the rate of histamine production by *Escherichia coli* in a medium containing only

inorganic salts and glycerol in addition to histidine. In their experiment, histamine formation took place very slowly; none was formed in 2 days; in 5 days, only 3.3 per cent of the available histidine had been converted to histamine; in 10 days, 24.4 per cent; and in 40 days, 83.5 per cent.

When a richer medium is used, histamine production is much more rapid, as is shown in table 1.

Similar experiments with other organisms and other media show that on the richer media considerable histamine is formed

TABLE 1

Rate of the production of histamine by Shigella dysenteriae St. on medium 5 (meat extract-peptone-histidine-glucose) at 31°C.

	HISTAMINE PRODUCED (AS THE DIHYDROCHLORIDE)	HISTIDINE CONVERTED TO HISTAMINE
	mgm. per cc.	*per cent*
1-day culture	0.094	11.6
2-day culture	0.191	23.6
5-day culture	0.417	51.6
11-day culture	0.444	55.0
15-day culture	0.463	57.3
23-day culture	0.460	56.9
30-day culture	0.458	56.7

during the first 24 hours; that most of the production takes place within 5 days, and that the maximum yield is obtained in about 2 weeks.

II. THE EFFECT OF THE pH ON THE PRODUCTION OF HISTAMINE

The effect of the pH was tested in a variety of ways. The most satisfactory procedure was as follows: A small amount of glucose (0.3 per cent) was added to the medium. Eight to 10 hours after inoculation, when active growth had started, the cultures were adjusted to the desired pH by the addition of sterile 10 per cent acetic acid or 4 per cent sodium hydroxide. During the first 48 hours, the pH of a culture changes rapidly, and the pH must be adjusted every 6 to 8 hours; after that, two or even one daily adjustment suffices. To determine the pH,

large loopfuls of the cultures were mixed with drops of indicator on a spot plate; the colors were then compared with those produced in a similar way by known buffer solutions.

The results thus obtained with several histamine-producing organisms are shown in table 2. This table shows that, with two exceptions, the maximum yields of histamine are obtained at pH 5.0 to 5.5. In no case (except with *Aerobacter aerogenes*) was there appreciable histamine formation at a pH more alkaline than 6.2.

TABLE 2

The effect of the pH on the histamine production on medium 5 (meat extract-peptone-histidine, but with 0.3 per cent glucose)

ORGANISM	TEMPERATURE OF INCUBATION	pH 4.5	pH 5.0	pH 5.3	pH 5.5	pH 5.7	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0
	°C.										
Escherichia coli Sta.	26	0.008	0.016	0.016	0.021	0.037	0.070	0.013	0	0	0
Escherichia coli Ev.	31	0.107	0.168	0.053	0.044	0.032	0.010	0.002	0	0	0
Escherichia coli Har.	37	0.065	0.123	0.098	0.098	0.068	0.020	0.005	0	0	0
Salmonella schottmuelleri Old.	31	0.157	0.389	0.364	0.304	0.276	0.065	0.034	0	0	0
Shigella dysenteriae St.	31	0.357	0.432	0.448	0.245	0.242	0.245	0.020	0	0	0
Shigella paradysenteriae Rya.	31	0.061	0.133	0.157	0.120	0.088	0.073	0.020	0	0	0
Shigella alkalescens Kau.	31	0.123	0.266	0.197	0.187	0.136	0.081	0.015	0	0	0
Eberthella typhi Mt. S.	37	0.107	0.264	0.357	0.352	0.243	0.165				
Aerobacter aerogenes Hul.	26	0.136	0.211	0.485	0.501	0.488	0.485	0.485	0.488	0.272	0.048

The results are recorded as mgm. of histamine di-hydrochloride per cubic centimeter of culture. The time of incubation was 14 days. The temperatures selected were those that are optimal for histamine production by that organism.

The two organisms in table 2 that gave unusual results are *Escherichia coli* Sta. and *Aerobacter aerogenes* Hul. *E. coli* Sta., which is a very efficient producer of histamine on other media (table 3) has consistently given low values for the meat-extract-peptone-histidine-glucose medium, which apparently contains some inhibitory factor which may also be responsible for the somewhat atypical pH effect. *Aerobacter aerogenes* Hul., and other histamine producers of this species, differ from all other histamine-forming organisms in being uninfluenced by the pH over a wide range. Only at the extremes (pH 5.0 to 5.5, and 7.5 to 8.0) is there any decrease in histamine production.

Koessler and Hanke (1919b) concluded, from their study of

Escherichia coli, that an acid reaction was indispensable for histamine production. Bertrand and Berthelot (1913), Mellanby and Twort (1912–13) and Jones (1918), on the other hand, have described organisms which produce histamine in alkaline reactions only, never in the presence of glucose. A search was made for such organisms, using the technic employed by these authors, but without success. As Jones succeeded only once in 50 attempts, such organisms cannot be common.

III. THE EFFECT OF THE TEMPERATURE AND THE COMPOSITION OF THE MEDIUM ON HISTAMINE PRODUCTION

Table 3 shows the effect of these two factors on histamine production by several representative organisms.

Temperatures above 37°C. depress histamine formation by all of the bacteria tested. In most cases, temperatures of 26°C. or less are also unfavorable. With some organisms (such as *Salmonella enteriditis* V and some not given in table 3) variations between 37°C and 26°C. have very little effect. In most instances, however, the temperature effect is very striking. An extreme case is that of *Escherichia coli* Sta., which produces over 40 times as much histamine at 26°C. as at 37°C. on the ammonium-nitrate-histidine-glucose medium. Differences of 3- or 4-fold in the histamine yield with a variation of only a few degrees of temperature are common.

Different species and even different strains of the same species respond differently to temperature variations. Thus, most strains of *Escherichia coli* have their optimum for histamine production at 31° to 26°C., but others at 37°C. (such as *Escherichia coli* Har., table 3). All of the *Salmonella* strains studied have their optimum at 34° to 31°C.; 7 strains of *Eberthella typhi* at 37° to 34°C.; 4 strains of *Shigella* at 31° to 26°C.; 5 strains of *Aerobacter aerogenes* at 26°C.; *Clostridium welchii* and *Bacteroides varius* at 37° to 34°C.; and *Bacteroides ovatus* at 31°C.

The composition of the culture fluid has a marked effect upon histamine production, as has been recognized by Bertrand and Berthelot (1913) and by Hanke and Koessler (1922). In these

TABLE 3

Effect of the temperature and the composition of the medium on histamine production

ORGANISM	TEMPERATURE	MEDIUM 1. AMMONIUM-NITRATE-HISTIDINE-GLUCOSE	MEDIUM 2. ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 3. ASPARAGINE-HISTIDINE-CYSTEINE-GLUCOSE	MEDIUM 4. YOLK INFUSION-ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 5. MEAT EXTRACT-PEPTONE-HISTIDINE-GLUCOSE	MEDIUM 6. MEAT EXTRACT-PEPTONE-HISTIDINE-GLYCEROL
	°C.						
Escherichia coli Sta.	41				0.009	0.011	
	37	0.010	0.032	0.157	0.055	0.046	0.537
	34	0.185	0.331	0.840	0.480		
	26	0.425	0.451	0.629	0.637	0.126	0.633
	20	0.041	0.440	0.580	0.522	0.078	0.118
Escherichia coli Har.	41			0.013	0.025	0.021	
	37	0.003	0.017	0.058	0.175	0.166	0.033
	31			0.032	0.058	0.141	
	26	0.002	0.001	0.023	0.029	0.110	
Escherichia coli Tem.	37	0.014	0.104	0.007	0.267	0.124	
	31	0.061	0.216	0.065	0.492	0.151	0.041
	26	0.064	0.272	0.065	0.645	0.197	
Salmonella schottmuelleri Old.	37	0.002	0.001	0.014	0.138	0.336	0
	34	0.003	0.005	0.012	0.268	0.288	0
	31	0.003	0.009	0.021	0.264		
	26	0.004	0.011	0.016	0.305	0.240	0
Salmonella enteriditis V.	37	0	0	0.007	0.200	0.135	0
	34	0	0	0.008	0.211	0.129	0.010
	31	0	0	0.007	0.352	0.236	0.015
	26	0	0	0.009	0.261	0.196	
Eberthella typhi Mt. S.	41	No growth			0.027	0.111	
	37				0.298	0.303	0.010
	34				0.341	0.316	0.008
	31				0.200	0.283	0.005
	26				0.099	0.181	
Shigella dysenteriae St.	37	0	0.007	0.016	0.267	0.234	0.022
	34			0.026	0.481	0.362	
	31	0	0.034	0.037	0.498	0.403	0.003
	26			0.035	0.391	0.411	

TABLE 3—*Concluded*

ORGANISM	TEMPERATURE	MEDIUM 1. AMMONIUM-NITRATE-HISTIDINE-GLUCOSE	MEDIUM 2. ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 3. ASPARAGINE-HISTIDINE-CYSTEINE-GLUCOSE	MEDIUM 4. YOLK INFUSION-ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 5. MEAT EXTRACT-PEPTONE-HISTIDINE-GLUCOSE	MEDIUM 6. MEAT EXTRACT-PEPTONE-HISTIDINE-GLYCEROL
	°C.						
Shigella paradysenteriae Rya.	37	0.013	0.069	0.076	0.560	0.133	0.388
	31	0.080	0.347	0.399	0.587	0.111	0.356
	26	0.102	0.475	0.644	0.651	0.230	
Shigella alkalescens Kau.	37	0.024	0.049	0.104	0.352	0.060	0.590
	31	0.072		0.544	0.803	0.136	0.451
	26			0.651	0.597	0.206	0.060
Aerobacter aerogenes Hul.	41			0	0	0	0
	37	0.001	0.215	0.092	0.011	0.015	0.304
	31	0.025	0.381	0.500	0.512	0.405	0.325
	26	0.042	0.466	0.582	0.619	0.243	0.284
	20	0.003	0.485	0.419	0.275		
Clostridium welchii Ti.	37	No growth			0.165*	0.391	
	31				0.157*	0.251	
Bacteroides ovatus	37				0.076*	0.091	
	31				0.296*	0.267	

Results are expressed in milligram of histamine di-hydrochloride per cubic centimeter of culture. The time of incubation was 14 days.

* Cysteine hydrochloride was added to this medium in a concentration of 1:1000.

experiments, the lowest yields usually occurred with the ammonium-nitrate-histidine-glucose medium. This is partly explained by the fact that growth here is often sparse or lacking; the pH attained is usually 5.6 to 6.0, which is not optimal. With this medium, a small quantity of imidazol bases other than histamine may appear in the acid extract (see table 6).

When an organic compound of nitrogen, such as asparagine, is supplied in addition to the histidine, the histamine yield is usually increased. Such an increase was observed by Hanke and

Koessler (1922) with several amino acids. This increase is in part due to the more favorable pH obtained (pH 5.0 to 5.5 when glucose is present). In many cases, the addition of cysteine to the asparagine medium still further increases histamine production; with one organism, however (*Escherichia coli*, Tem.), the addition of cysteine greatly diminished the yield.

When egg-yolk infusion was added to the medium, histamine production was in most cases greatly stimulated. In many instances, especially with the anaerobes and with all strains of *Eberthella typhi*, the addition of cysteine still further augmented the yield of histamine.

The meat-extract-peptone-histidine-glucose medium was rather variable in its effect on histamine production. In many cases, it was as good or better than other media; while with other organisms it gave comparatively low yields.

When glycerol was used instead of glucose, as in the meat-extract-peptone-histidine-glycerol medium, the production of histamine was usually very low. This is probably due to the fact that these organisms produce little or no acid from glycerol, hence the pH remains too alkaline for histamine production. But in 5 instances (3 strains of *Escherichia coli*, *Shigella paradysenteriae* Rya., and *Shigella alkalescens* Kau.) the production of histamine was considerably increased when glycerol was substituted for glucose (tables 3 and 4). Also, with *Aerobacter aerogenes*, there was a marked difference in favor of the glycerol, but only at the higher temperatures. With the 5 strains mentioned above, the pH with the glycerol medium ran between 5.7 and 6.2, reactions which one would expect to be less favorable for histamine production than the pH 5.0 to 5.3 obtained on the glucose medium. The results indicate that for these strains at least, the carbohydrate has some other effect than that of stimulating growth and providing a favorable acidity.

The addition of 5 per cent of serum to these media made very little difference in the histamine yield. As a rule, the addition of serum slightly diminished histamine production; in a few cases, it slightly increased the yield.

TABLE 4

Production of histamine by bacteria on media of different composition

ORGANISM	TEMPERATURE	MEDIUM 1. AMMONIUM-NITRATE-HISTIDINE-GLUCOSE	MEDIUM 2. ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 3. ASPARAGINE-CYSTEINE-HISTIDINE-GLUCOSE	MEDIUM 4. YOLK INFUSION-ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 5. MEAT EXTRACT-PEPTONE-HISTIDINE-GLUCOSE	MEDIUM 6. MEAT EXTRACT-PEPTONE-HISTIDINE-GLYCEROL
	°C.						
Escherichia coli Sta.*	26	0.425	0.452	0.629	0.637	0.126	0.633
Escherichia coli Har.*	37	0.003	0.017	0.058	0.175	0.166	0.033
Escherichia coli O'L	26	0.002	0.002	0.003	0.019	0.097	0.005
Escherichia coli Sch	26	0	0.003	0.012	0.044	0.088	0
Escherichia coli Tem.*	26	0.064	0.272	0.065	0.645	0.197	
Escherichia coli Mal	31	0	0	0	0.025	0.053	0
Escherichia coli Ort.*	37	0	0	0	0.002	0.154	0.268
Escherichia coli Kie	37	0	0.067	0.106	0.121		
Escherichia coli Bro.*	37	0	0.020	0.016	0.205	0.107	0.120
Escherichia coli Rak	37	0	0.003	0.005	0.027		
Escherichia coli Pen	26	0	0.002	0.002	0.072	0.273	0.039
Escherichia coli Ev	26	0	0.001	0.006	0.024	0.188	0.030
Escherichia coli Jon	26	0	0.007	0.006	0.051		
Escherichia coli H 1	34				0.307	0.128	
Escherichia coli H 2	34			0	0.041	0.110	
Escherichia coli Ton	34				0.213		
Escherichia coli McC	34				0.053		
Escherichia coli Mer	34				0.212		
Salmonella paratyphi St	31	0	0.002	0.027	0.109		
Salmonella paratyphi Frl.*	31	0	0	0.009	0.267	0.142	0.016
Salmonella schottmuelleri Old.*	34	0.003	0.005	0.012	0.268	0.288	0
Salmonella schottmuelleri 222*	31			0.007	0.352	0.324	0.006
Salmonella schottmuelleri Rak.*	31	0	0	0	0.190		
Salmonella enteriditis V.*	31	0	0	0.007	0.352	0.236	0.015
Salmonella enteriditis McK	31			0.009	0.101		
*Salmonella suis**	31			0.002	0.147	0.306	0.004
*Salmonella aertrycke**	31			0	0.152	0.203	0.017
Eberthella typhi Pfe.*	37	No growth			0.087	0.151	0
Eberthella typhi Sch.*	37				0.188	0.090	0.005
Eberthella typhi Mt. S.*	37				0.298	0.303	0.010
Eberthella typhi Mor	37				0.064	0.082	0
Eberthella typhi Spa	37				0.009	0.115	0
Eberthella typhi Bra	37				0.007	0.129	0
Eberthella typhi Dan.*	37				0.090	0.200	0.002

* Crystals of histamine di-picrate, m.p. 238–241°C. were prepared from the acid extracts.

TABLE 4—*Concluded*

ORGANISM	TEMPERATURE	MEDIUM 1. AMMONIUM-NITRATE-HISTIDINE-GLUCOSE	MEDIUM 2. ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 3. ASPARAGINE-CYSTEINE-HISTIDINE-GLUCOSE	MEDIUM 4. YOLK INFUSION-ASPARAGINE-HISTIDINE-GLUCOSE	MEDIUM 5. MEAT EXTRACT-PEPTONE-HISTIDINE-GLUCOSE	MEDIUM 6. MEAT EXTRACT-PEPTONE-HISTIDINE-GLYCEROL
	°C.						
Shigella dysenteriae St.*	31	0	0.034	0.037	0.498	0.403	0.003
Shigella paradysenteriae Rya.*	26	0.102	0.475	0.644	0.651	0.230	0.356
Shigella paradysenteriae Son.	26	0	0	0	0.097	0.047	
Shigella alkalescens Kau.*	31	0.072		0.544	0.803	0.136	0.451
Aerobacter aerogenes Hul.*	26	0.042	0.466	0.582	0.619	0.243	0.284
Aerobacter aerogenes Woo.*	26	0.093			0.338	0.668	0.700
Aerobacter aerogenes Sm	26				0.041	0.020	0.078
Aerobacter aerogenes Mc	26			0.027	0.141	0.184	0.067
Aerobacter aerogenes El	31				0.272	0.144	0.034
Clostridium welchii Ti.*	37	No growth			0.165†	0.391	
Clostridium welchii Eg.	34				0.120†	0.251	
Clostridium welchii Br.	34				0.120†	0.167	
Clostridium welchii Ca.	34				0.162†	0.275	
*Bacteroides ovatus**	31				0.296†	0.275	
*Bacteroides varius**	37				0.022†	0.256	

† Cysteine hydrochloride was added to a concentration of 1:1000. Results are expressed in milligram of histamine di-hydrochloride per cubic centimeter of culture. The time of incubation was 14 days.

IV. THE ORGANISMS THAT WERE FOUND TO PRODUCE HISTAMINE

In table 4, all of the organisms that were found to produce histamine have been listed. Most of them were tested at several different temperatures; in this table, only the yields for one temperature are given. This was not always the optimum, as the optimum temperature was not determined in every case.

One is impressed by the fact that every organism in table 4 has its habitat in the intestinal tract; not one non-intestinal organism produced histamine. The converse, however, is not true, for many species of intestinal bacteria do not produce histamine.

Every strain of *Escherichia coli* that was tested produced

some histamine. This was likewise true of every strain of the genus *Salmonella* and of every strain of *Eberthella typhi*. On the other hand, of the 8 strains of *Shigella* that were investigated, only the 4 given in table 4 were positive; and of 14 strains of *Aerobacter aerogenes*, only 5 produced histamine. Four strains only of *Clostridium welchii* were studied; all were positive. Only one strain each of *Bacteroides ovatus* and *Bacteroides varius* were available.

V. HISTAMINE PRODUCTION BY "RESTING" BACTERIA

Medium 7, containing only histidine and salts, was employed in these experiments. As little or no growth can take place in this fluid, very heavy inoculations were made. Young agar cultures were washed twice with sterile distilled water, and enough organisms were added to the medium to give a density of about 2 billion bacteria per cubic centimeter.

TABLE 5

Histamine production by resting bacteria

ORGANISM	HISTAMINE DI-HYDROCHLORIDE PRODUCED	HISTIDINE CONVERTED
	mgm. per cc.	*per cent*
Eschericha coli Sta	0.694	85.9
Escherichia coli Har	0	0
Salmonella schottmuelleri 222	0	0
Eberthella typhi Mt. S	0	0
Shigella dysenteriae St	0	0
Shigella paradysenteriae Rya	0.437	54.1
Shigella alkalescens Kau	0.778	96.3
Aerobacter aerogenes Hul	0	0

The temperature was 34°C., and the time of incubation was 7 days.

In one experiment (table 5) the medium was brought to pH 5.2 with acetic acid; it is therefore well buffered at this pH.

All of the organisms shown in table 5 will produce abundant histamine on other media. Yet only 3 of these 8 organisms formed histamine under these conditions; the same 3 will form histamine in the ammonium-nitrate-histidine-glucose medium. The 5 negative organisms of table 5 partially converted the

histidine to another base that appeared in the acid extracts; this base does not contain the imidazol nucleus, for only a pale yellow color is produced when it is coupled with sulfanilic acid. It is apparently identical with a base encountered by Koessler and Hanke (1919b) to which they ascribed the formula $HCNH_2:CNH_2CH_2CH_2NH_2$. Imidazol bases other than histamine did not appear in the acid extracts.

VI. THE PRODUCTION OF IMIDAZOL BASES OTHER THAN HISTAMINE

As stated before, imidazol bases other than histamine appeared in the acid extracts only when the cultures were made on medium

TABLE 6

Production of histamine and of extractable imidazol bases other than histamine on the ammonium-nitrate-histidine-glucose medium

ORGANISM	HISTAMINE	IMIDAZOL BASE, NOT HISTAMINE
Escherichia coli Sta	0.299	0.025
Escherichia coli Kier	0	0.012
Escherichia coli Tem	0.061	0.012
Shigella paradysenteriae Rya	0.057	0.015
Shigella alkalescens Kau	0.072	0.016

The time of incubation was 14 days; the temperature, 31°C.

The results in both columns are expressed as histamine di-hydrochloride in milligrams per cubic centimeter.

1. In this medium, ammonium salts and nitrates are the only sources of nitrogen beside the histidine, and glucose is present. When the amount of these bases is small (as is invariably the case), they will not be precipitated from the acid extracts by phosphotungstic acid (Eggerth, Littwin, and Deutsch, 1939), whereas the histamine will be completely removed. If the precipitate is dissolved in sodium carbonate solution, and the supernatant fluid is neutralized, both fractions can be assayed with the diazo reagent in the usual way. The results of several such tests are given in table 6.

This table shows that the total amounts of non-histamine imidazol bases in the acid extract were always small. For

this reason, no attempt was made to isolate and identify them. When treated with the diazo reagent, their color was redder than that of the histamine standard, suggesting that they might be methyl imidazol or some homologue such as ethyl or vinyl imidazol.

VII. ORGANISMS THAT DO NOT PRODUCE HISTAMINE

The following bacteria were tested on two or more media each, and at two different temperatures, and were found negative for histamine.

	Number of strains
Proteus vulgaris	6
Alcaligenes fecalis	2
Shigella paradysenteriae	4
Aerobacter aerogenes	9
Klebsiella pneumoniae	3
Klebsiella ozaenae	1
Klebsiella rhinoscleromatis	1
Vibrio comma	1
Vibrio metchnikovi	1
Brucella abortus	1
Brucella melitensis	1
Pseudomonas aeruginosa	2
Neisseria intracellularis	3
Neisseria gonorrheae	2
Neisseria flava	1
Neisseria catarrhalis	1
Neisseria crassus	1
Streptococcus hemolyticus	3
Streptococcus viridans	1
Enterococcus	6
Diplococcus pneumoniae	3
Staphylococcus aureus	3
Corynebacterium diphtheriae	1
Bacillus subtilis	2
Bacillus mycoides	1
Bacillus mesentericus	1
Bacillus megatherium	1
Lactobacillus acidophilus	2
Clostridium tetani	2
Clostridium putrificum	1
Clostridium histolyticum	1
Clostridium bifermentans	1
Bacteroides bifidus	4
Bacteroides pseudoramosus	3

	Number of strains
Bacteroides aerofaciens	3
Bacteroides biformis	3
Bacteroides avidus	2
Bacteroides limosus	1
Bacteroides catenaformis	2
Bacteroides lentus	3
Bacteroides gulosus	3
Bacteroides thetaiotaomicron	3
Bacteroides variabils	3
Bacteroides uniformis	3
Bacteroides vulgatus	4
Bacteroides distasonis	3
Bacteroides exiguus	1
Bacteroides vescus	1
Bacteroides insolitus	1

Where organisms did not grow well on the usual media, special media were used. Thus, 5 per cent of serum was added to cultures of the *Neisseria*, *Streptococcus*, and *Diplococcus* genera; and *Lactobacillus acidophilus* was cultivated in a milk histidine medium.

DISCUSSION

The only other investigations in which a large number of strains and species of organisms were studied have been those of Hanke and Koessler (1922) and of Koessler, Hanke, and Sheppard (1928). Hanke and Koessler studied 62 strains (29 of these were *Escherichia coli*) on a medium containing only histidine, inorganic salts, and glycerol. Six of these strains—all of them *Escherichia coli*—formed histamine. No positive results were obtained with any member of the *Salmonella*, *Eberthella*, or *Shigella* genera. Koessler, Hanke, and Sheppard studied 223 strains from 94 species on a medium containing meat extract, peptone, histidine, whole blood, and glycerol. (These authors preferred glycerol to glucose because, when glucose was used, troublesome interfering substances were extracted by their method.) In this investigation, 9 histamine producing organisms were found; 2 were *Escherichia coli*, 1 was a *Salmonella enteriditis*, 1 was a *Salmonella schottmuelleri*, and 5 were *Salmonella morgani*. In the same series, 7 other strains of *Escherichia coli* and 42

other strains of *Salmonella* were negative, as were also all strains of *Eberthella*, *Shigella*, and *Aerobacter*.

In the present investigation, histamine was formed by every one of the 18 strains of *Escherichia coli* tested; likewise every one of 9 strains of *Salmonella* (from 5 species), and every one of 7 strains of *Eberthella typhi*. Four out of 8 strains of *Shigella* were positive, as well as 5 out of 14 strains of *Aerobacter aerogenes*. Organisms from all of these genera were active producers of histamine, often converting from 30 to 100 per cent of the histidine to the amine. There can be no doubt that the color-producing substance formed by these organisms actually was histamine, as the crystalline di-picrates were prepared in most cases from the acid extracts (table 4).

There are several reasons why many more histamine-positive organisms were found than in the above mentioned investigations. The most important one is that glucose was employed in the media instead of glycerol. When glucose is supplied, the favorable pH of 5.0 to 5.5 is usually obtained, whereas most organisms do not produce enough acid from glycerol to permit histamine formation. Tables 3 and 4 show the striking effect of substituting glucose for glycerol.

Very often the temperature of incubation is an important contributory factor. Hanke and Koessler (1922) and Koessler, Hanke, and Sheppard (1928) incubated their cultures at 37.5°C., which is not, in most cases, the optimal temperature for histamine production.

Hanke and Koessler (1922) employed a medium that contained no organic source of nitrogen except histidine itself. Tables 3 and 4 show that such a medium is usually unfavorable to histamine production, and that very few organisms will be positive when it is used.

SUMMARY

1. Using a simplified technic previously described (Eggerth, Littwin, and Deutsch, 1939) histamine production has been determined in cultures of 49 strains of bacteria, belonging to 14 species of 7 genera. These organisms all have their habitat in the intestinal tract.

2. With the exception of *Clostridium welchii*, all of these organisms require free histidine for histamine formation.

3. In a favorable medium, histamine production begins within 24 hours and continues rapidly for 4 to 5 days, after which the rate of production decreases.

4. For most organisms, the optimal pH for histamine production is pH 5.0 to 5.5, and no histamine is produced at reactions more alkaline than pH 6.5. However, the histamine forming strains of *Aerobacter aerogenes* will produce this amine at any pH between 5.0 and 8.0.

5. The temperature of incubation markedly affects histamine formation. Temperatures higher than 37°C. and lower than 26°C. are usually unfavorable. Between these limits, the optimal temperature varies a great deal with different organisms.

6. The yield of histamine is determined also by the composition of the culture medium. Where only inorganic compounds of nitrogen are supplied, in addition to histidine, the yields of histamine are usually low, and some of the histidine may be converted to other imidazol bases. The addition of amino acids, such as asparagine and cysteine, or of peptone, or of egg yolk or meat infusion, increases histamine production. The nature of the added carbohydrate is also important, chiefly because of the effect of the pH of the culture.

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