TABLE I.

Host	Species of flea
M. californicus	*Catallagia vonblockeri* (Augustson, 1941) *Atyphloceras multidentatus* (C. Fox, 1909) *Hystrichopsylla gigas dippiei* (Rothschild, 1902) *Malaraeus telchinum* (Rothschild, 1905) *Peromyscopsylla ebrighti* (C. Fox, 1926)
P. maniculatus	*Atyphloceras multidentatus* *Hystrichopsylla gigas dippiei* *Opisodasys nesiotus* (Augustson, 1941) *Malaraeus telchinum* *Catallagia vonblockeri*
R. norvegicus	*Malaraeus telchinum* *Nosopsyllus fasciatus* (Bose, 1801) *Orchopeas sexdentatus* (Fox, 1909)
R. megalotis	*Catallagia wymani* (Fox, 1909)

with salt solution, and inoculated into a guinea pig which died on the 8th day with lesions of plague. A *Microtus* was placed in the crock with the remaining 69 fleas. The animal died in 13 days with the anatomical findings and the cultural isolation of *P. pestis*.

(2) One hundred *Malaraeus telchinum* were placed on a *Microtus* with a terminal plague septicemia; the rodent died within a few hours. Three days later a *Microtus* placed in the crock with the fleas contracted plague and died on the 15th day after exposure.

(3) Seventy-five fleas were placed on a white laboratory mouse with many plague bacilli in the blood. Twenty-four hours after the death of the mouse, a *Microtus* was exposed in the crock. Six days later it was found dead from plague.

(4) An identical experiment was conducted with 25 *Malaraeus*. The *Microtus* contracted plague and died on the 6th day after exposure. Experiments to explain the earlier failures and to evaluate the vector efficiency are in progress.

The valuable assistance of Mr. Robert Holdenried in collecting the specimens and in making the specific determinations of the mammals, and of Dr. M. A. Stewart with the flea identifications is gratefully acknowledged.

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On the Specificity of Bacterium-Decarboxylase.

E. GEIGER.

From the VanCamp Laboratories, Terminal Island, Calif.

In a recent study[1] on the histamine-formation in fish muscle it was found that a minor fraction of histamine-units produced post mortem by bacterial action remains within a protein complex and can be liberated only by acid hydrolysis or by fermentative proteolysis. It was assumed, therefore, that histidine groups need not be present in a free form in order to be converted into histamine groups. This implies that some bacterial ferments are able to split such peptide-linkages which involve the carboxyl group of histidine groups without attacking simultaneously those linkages by which the histamine group is attached to the protein molecule. The possible formation of such compounds was discussed by Guggenheim[2] who assumed on a theoretical

[1] Geiger, E., Courtney, G., and Schnakenberg, *Arch. Biochem.*, in press.

[2] Guggenheim, M., *Biochem. Z.*, 1913, **51**, 369.

basis that "bacterial or fermentative processes which produce amines from aminoacids may also convert polypeptides into peptamines." In view of some recent experiments by Gale[3] in which a high specificity of bacterial decarboxylases was demonstrated, it was desirable to investigate the possibility of bacterial decarboxylation of some histidine derivatives. Since the hydrolytic action of bacterial peptidases coincides with the decarboxylation of di- and polypeptides it was investigated first whether histidine derivatives containing monoacylated aminogroups like benzoyl- or acetylhistidine can be decarboxylated by bacteria.

Methods. The benzoylhistidine (1α-benzoylamino-β-imidazolyl-4(5)-propionic acid) and benzoylhistamine (N-β-imidazolyl-4(5)-ethyl-benzamid) were prepared according to Gerngross,[4] and had a melting point 247° and 148°C (cor) respectively. Our monoacetyl histidine sample was obtained according to Bergmann and Zervas[5] (m.p. 169°C cor.). Acetyl-histamine was prepared from free histamine using the same method; a pale yellow solid was obtained, which until now did not crystallize. The decarboxylation was performed according to Gale,[3] however lower concentrations of the substrate were added than in Gale's experiments since benzoylhistidine is less soluble at acid reaction than histidine. Each set of experiments was performed with 3 different strains of *Escherichia coli,* cultured from human feces. It was shown in control experiments that all 3 strains were strong histamine-formers, viz., they decarboxylated added histidine almost quantitatively. A culture of *E. coli* in 500 ml of tryptose broth (pH 5.0) was incubated at 30°C for 16 hours, centrifuged and washed twice with sterile saline solution. The bacteria were then suspended in 10 ml of the saline. To this suspension we added 10 ml of phthalate-buffer (pH 5.0) and 50 mg of benzoylhistidine or acetylhistidine in 30 ml of water. A blank was prepared in the same way but contained 10 ml of water instead of the bacterial suspension. The samples were incubated for 6 to 12 hours at 37°C. The biological activity was assayed on the isolated guinea pig intestine, suspended in 20 ml of Locke-Ringer solution.

Results. After incubating acetyl- or benzoylhistidine with the bacteria even for 12 hours, the ninhydrin reaction, which would indicate the presence of free histidine, remained negative. Consequently *E. coli contains no acylases which would be able to split acetyl- or benzoylhistidine.* The mentioned reaction became however strongly positive after hydrolysing the sample with 5% HCl for one hour.

It was then investigated whether or not acylated histamines can be produced by *E. coli* from acetyl- or benzoylhistidine by decarboxylation. After 12 hours incubation no histaminelike activity on the guinea pig ileum was detected. The same intestinal strip responded to 0.05-0.1 γ of histamine. The failure to contract could be attributed either to the inability of the bacteria to decarboxylate the acyl compounds or to the pharmacological inactivity of the decarboxylated compounds. Therefore the action of benzoyl- and acetylhistamine was studied and it was found that they were without effect even in concentrations which were 100,000 times higher than the minimum effective histamine-concentration. After hydrolysing the solution of benzoyl- or acetylhistamine with 10% HCl on a boiling waterbath for one hour the solution had the expected very strong action on the intestine due to the formation of the free base.

On the basis of these experiments it seemed possible that during the incubation with bacteria the acylated histidines were decarboxylated to give biologically inactive acylhistamines. The validity of this assumption was tested by subjecting the incubated solution to acid hydrolysis, after which however no biological activity was found. Such a hydrolysis would liberate active histamine from its inactive acetyl- or benzoyl-derivatives. We claim therefore *that during the incubation with bacteria no benzoyl- or acetyl-*

[3] Gale, F., *Biochem. J.*, 1940, **34**, 393; 1941, **35**, 67.

[4] Gerngross, O., *Z. f. physiol. Chem.*, 1920, **108**, 50.

[5] Bergmann, M., and Zervas, L., *Biochem. Z.*, 1928, **280**, 203.

histamine was produced from the corresponding histidine derivatives.

That the bacterial decarboxylase is not inhibited by acyl-histidines was shown by the following experiments:

Sample A: Bacterial suspension + 50 mg histidine.

Sample B: Bacterial suspension + 50 mg histidine + 50 mg acetylhistidine.

Sample C: Bacterial suspension + 50 mg acetylhistidine.

After 12 hours incubation only Sample C had no activity on the intestine, while Samples A and B contained equal amounts of histamine (35-40 mg), indicating that presence of acetylhistidine does not inhibit the decarboxylation of histidine. Analogous results were obtained with benzoylhistidine.

Summary. The reported experiments show that the ability of *E. coli* to decarboxylate histidine and its derivatives is highly specific, since the acylation of the amino group hinders this process. We cannot decide however whether or not the *E. coli* are able to convert peptides into "peptamines" by decarboxylation. If a free amino group should be necessary also in peptides, then such amino group could be provided not only by histidine but also by peptides containing histidine units with free carboxyl groups.

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Effect of Low Potassium Diet and Desoxycorticosterone on the Rat Heart.*

DANIEL C. DARROW. (Introduced by Grover F. Powers.)

From the Department of Pediatrics, Yale University School of Medicine.

In a previous publication[1] attention was directed to lesions produced in the heart by injections of desoxycorticosterone acetate. This was to be expected since heart lesions develop on diets low in potassium[2,3,4] and injections of desoxycorticosterone acetate produce a deficit of potassium and changes in muscle composition similar to those accompanying diets low in potassium.[1,5,6] The lesions in the heart are not necessarily accompanied by changes in the electrolyte composition of the heart, and in rats it has not been certain that loss of heart potassium is produced either by diets low in potassium or by injections of desoxycorticosterone acetate. Study of the effects of the combination of a diet low in potassium together with injection of desoxycorticosterone acetate was, therefore, undertaken to determine if heart potassium can be reduced in rats and if lesions are more quickly produced by this means.

The chemical methods and the low potassium diet were described in previous studies.[7] Owing to the size, the hearts of several animals were pooled and analyzed as a whole. Sufficient material was not available to include fat and chloride analyses. All values are expressed per 100 g of dried tissue.

Table I shows the results. While neither the diet low in potassium nor injections of desoxycorticosterone give more than a questionable lowering of heart potassium in 30 days, the combination of the diet low in potassium

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1 Darrow, D. C., and Miller, H. C., *J. Clin. Invest.*, 1942, **21**, 601.

2 Schrader, G. A., Pricket, C. O., and Salmon, W. D., *J. Nutrition*, 1937, **14**, 85.

3 Follis, R. H., Oerent-Keiles, E., and McCollum, E. V., *Am. J. Path.*, 1942, **18**, 29.

4 Thomas, R. M., Mylon, E., and Winternitz, M. C., *Yale J. Biol. and Med.*, 1940, **12**, 345.

5 Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchley, D. W., and Loeb, R. F., *Am. J. Physiol.*, 1941, **135**, 230.

6 Heppel, L. A., *Am. J. Physiol.*, 1939, **127**, 385.

7 Miller, H. C., and Darrow, D. C., *Am. J. Physiol.*, 1940, **130**, 747.