is continuously going on in the insect cell is illustrated in the Table. In order to show this, insects previously labeled with $C^{14}$ leucine or $C^{14}$ uridine are transferred and allowed to grow in a nonradioactive diet. At regular intervals a definite number of them are withdrawn, washed, homogenized and deproteinized by adding 5% cold TCA. The precipitate was washed, plated and counted as described before. The results of the Table show a regular decline in the radioactivity in the acid insoluble precipitate until very little activity is retained by the insect. These results can only be interpreted on the basis of continuous breakdown of proteins by intracellular proteases and RNA by RNase in the cells of the insect.

The progressive loss of radioactivity in *Tribolium confusum* due to continuous degradation of proteins and RNA by intracellular proteases and RNase's respectively during the life cycle of the insect

| | Counts per min per mg of TCA - insoluble material | | | | | |
|---|---|---|---|---|---|---|
| | 8th day | 11th day | 14th day | 17th day | 20th day | 24–26th day |
| Protein labeled with $C^{14}$-leucine | 40–45 | 18–20 | 6–8 | 3–4 | very little | nil |
| RNA, labeled with $C^{14}$-uridine | 90–95 | 50–60 | 35–40 | 14–16 | 4–6 | practically nil |

The insects were fed radioactive diets containing either $C^{14}$-leucine or $C^{14}$-uridine for 7 days; then on 8th day the radioactivity in the acid insoluble material was determined; a definite number of the insects was removed to a jar containing non-radioactive diet. At regular interval the radioactivity retained by the insect was determined. For experimental details see the text.

In summary, from this study one can conclude that the insect *Tribolium confusum*, Duval, shows great variations in the activities of those enzymes involved in the synthesis and breakdown of proteins and nucleic acids during its entire life cycle. The results, obtained in the course of this investigation, further indicate that RNA synthesis precedes protein synthesis and the protein synthesis can be considered as an index of growth of the insect[7].

*Résumé.* Dans notre étude, l'incorporation de la leucine-$C^{14}$ dans les protéines et de l'uridine-$C^{14}$ dans le RNA chez le *Tribolium confusum* s'est révélée maximale durant la phase la plus active de croissance, et minimale durant la phase de pupe, pendant laquelle l'animal ne mange pas. La synthèse du RNA précède celle des protéines, tel que prévu. Nos résultats montrent aussi une continuelle dégradation des protéines par des protéases intra-cellulaires et du RNA par des RNases.

ANIMA DEVI, P. LINDSAY, and N. K. SARKAR

*Department of Biochemistry, Faculty of Medicine, Laval University, Quebec (Canada), October 22, 1962.*

[7] This work has been supported by a grant received from National Research Council of Canada.—We are grateful to Dr. A. LEMONDE of this department for providing us with the insect.

## Occurrence of 'Partially Acid-Fast' Cells in Cultures of Genus *Staphylococcus* and Genus *Micrococcus*[1]

The occurrence of 'partially acid-fast' (PAF) cells in cultures of *Escherichia coli* was noted previously[2]. The cells were demonstrated by a simple staining method[3], which resembles the MACHIAVELLO[4–7], CASTAÑEDA[8], and KÖSTER[9,10] stains.

These experiments were extended to Gram positive cocci, namely, genus *Staphylococcus* and genus *Micrococcus* growing on nutritive agar (Difco), free from any agents such as antibiotics.

Smears were prepared from cultures incubated at 37°C for 17 and 41 h, and cultures incubated at room temperature for four weeks. The 'partially acid-fast' stain revealed PAF positive (red stained) and PAF negative (blue stained) cocci in all cultures of both genera (Figure). The positive forms were always outnumbered by the negative forms.

It has been stated that the carbol fuchsin penetrates the dead cells of *Staphylococci* more easily than living cells[11]; however, division of PAF positively stained cells was clearly observed in these experiments (see illustration). This division sometimes leads to development of small microcolonies consisting of PAF positive cocci.

A total of 66 strains of *Staphylococci* and 62 strains of *Micrococci* were studied. In the *Staphylococci*, 87% were PAF positive and in the Micrococci, 38%. Thus, the *Staphylococci* are more apt to produce PAF positive forms.

Most of the cultures contained Gram negative cocci as well as Gram positive forms, and it was shown that the PAF character coincides with the Gram positive cocci. This observation was made by comparison of color photomicrographs taken from the same area of smears stained first by the PAF method and later by the Gram technique. Thus, the Gram positive cocci can now be subdivided on the basis of staining into PAF positive and PAF negative forms.

[1] These studies were done with the aid of the Medical Research Council of Canada. Grant MA-729, 1962.

[2] G. NOGRADY, XIIIth Meeting of the Canadian Public Health Association, Seigniory Club, Montebello (Québec, Canada), December 6th (1962).

[3] *PAF staining method:* Stain with alcalinized 0.2% basic fuchsin solution (w/v) for 5 min at room temperature. Rinse with distilled water. Dip in 5% acetic acid for 1 sec and rinse again. Counterstain with 5% aqueous methylene blue for 1 min (saturated solution in 95% ethyl alcohol). Blot and dry without rinsing. Basic fuchsin should be prepared daily. Alcalinize 50 ml basic fuchsin solution (23°C) with 3 drops of n/1 Sodium hydroxyde (which must be stored in polyethylene bottle to avoid silicate contamination).

[4] A. MACHIAVELLO, Zentralbl. Bakt. Abt. I. Orig. *139*, 291 (1941).

[5] A. W. STABLEFORTH and I. A. GALLOWAY, *Infectious Diseases of Animals* (Butterworth, 1959), vol. 1, p. 63.

[6] H. ZINSSER, F. FITZPATRICK, and H. WEI, J. exp. Med. *69*, 179 (1939).

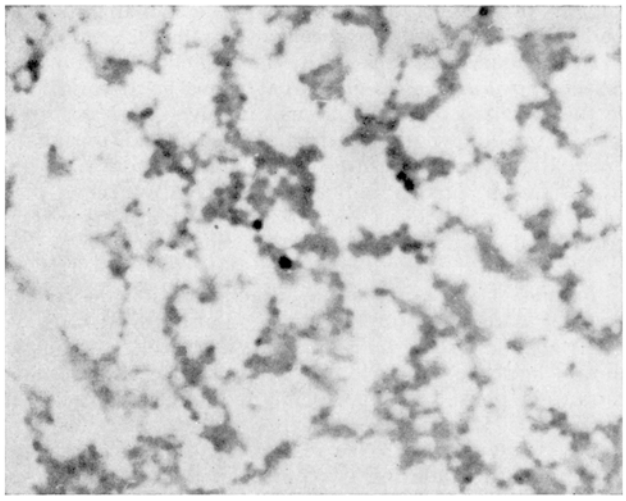
[7] P. F. ZDODOVSKII and H. M. GOLINEVICH, *The Rickettsial Diseases* (Pergamon Press 1960), p. 170.

[8] M. R. CASTAÑEDA and S. J. ZIA, J. exp. Med. *58*, 55 (1933).

[9] K. HANSEN and H. KÖSTER, Dtsch. tierärztl. Wschr. *44*, 739 (1936).

[10] H. KÖSTER in K. HANSEN and H. KÖSTER, Dtsch. tierärztl. Wschr. *44*, 739 (1936).

[11] S. D. ELEK, *Staphylococcus pyogenes and its Relation to Diseases* (Livingstone 1959), p. 50.



*Staphylococcus pyogenes* var. *aureus*, showing 'partially acid-fast' positive (dark) and 'partially acid-fast' negative (light) cells. On the right, 'partially acid-fast' positive cells just after division. Approx. 1600 ×.

Preliminary experiments were undertaken to try to establish a relationship between the PAF positive and PAF negative staining characters of *Staphylococci* and *Micrococci* to coagulase test, penicillin sensitivity (1 Unit/ml) and phage typability, but because of heterogeneity of results, no conclusions can be drawn at this time.

An attempt was made to elucidate this PAF character by comparing it with the classical acid-fast staining method of Ziehl-Neelsen. It was observed that in young cultures (17 h) of *Staphylococci*, the PAF character could be inactivated by previous treatment with 5% carbolic acid for 5 min. The same treatment does not affect the acid-fast stain of *Mycobacteria*; thus, this fact decisively separates the two staining characters.

Several factors remain unknown, including the effect of stimulating and inhibiting agents, the chemical or structural nature of PAF character and its relationship to viability and growth.

*Résumé.* Lorsqu'on applique aux populations des genres *Staphylococcus* et *Micrococcus* une coloration semblable à celle de MACHIAVELLO, on obtient des cellules différemment colorées.

Ce caractère «partiellement acido-résistant» diffère du caractère tinctorial de l'acido-résistance vraie.

G. NOGRADY

*Département de Bactériologie, Faculté de Médecine de l'Université de Montréal (Québec, Canada),*
*February 12, 1963.*

## Occurrence of New Imidazolealkylamines (Spinaceamine and 6-Methylspinaceamine) in Skin Extracts of *Leptodactylus pentadactylus labyrinthicus*[1]

In the course of a systematic study on biologically active amines in the amphibian skin, enormous amounts of histamine and related imidazolealkylamines were found in acetone extracts of the skin of *Leptodactylus pentadactylus labyrinthicus*, a South-American amphibian collected in Misiones (Argentina).

Chromatography on alkaline alumina column, paper chromatography, colour reactions and bioassay, carried out in parallel on the natural amines and on the corresponding synthetic compounds prepared by one of us (Vitali), have permitted the certain identification in the *Leptodactylus* extracts of the following imidazolealkylamines: histamine; 4(2-methylaminoethyl)-imidazole or N'-methylhistamine; 4(2-dimethylaminoethyl)-imidazole or N',N'-dimethylhistamine; 4,5,6,7-tetrahydroimidazo-[5,4-*c*]pyridine and 6-methyl-4,5,6,7-tetrahydroimidazo-[5,4-*c*]pyridine.

Owing to its strict relation to spinacine, the amino-acid discovered by ACKERMANN and MOHR[2] in the shark *Acanthias vulgaris* and by ACKERMANN[3] in the crab *Crango vulgaris*, we suggest the name *spinaceamine* for 4,5,6,7-tetrahydroimidazo[5,4-*c*]pyridine, and that of *6-methylspinaceamine* for its 6-methyl derivative.

The accompanying Table shows the Rf values of the above amines with two different solvent systems and the colour reactions produced on paper by the Pauly reagent and the Folin reagent for aminoacids.

Histamine, N'-methylhistamine and N',N'-dimethylhistamine displayed the well known potent stimulant effect on the guinea-pig ileum; spinaceamine and 6-methylspinaceamine were practically inactive[4].

[1] Supported by grants from the Consiglio Nazionale delle Ricerche, Roma, and the Rockefeller Foundation, New York.
[2] D. ACKERMANN and M. MOHR, Z. Biol. *98*, 73 (1936).
[3] D. ACKERMANN, Z. physiol. Chem. *328*, 275 (1962).
[4] G. BERTACCINI and T. VITALI, to be published.

| | Rf values | | Colour reactions | |
|---|---|---|---|---|
| | Methylethylketone + pyridine + water + 30% methylamine (65:15:10:0.5) | 1-Pentanol + pyridine + water + 30% methylamine (40:40:10:1) | Folin reagent | Pauly reagent |
| Histamine | 0.55 | 0.36 | grey-blue | pink red |
| N'-Methylhistamine | 0.50 | 0.53 | rose-pink | pink red |
| N',N'-dimethylhistamine | 0.68 | 0.67 | ? (pale pink) | pink red |
| Spinaceamine | 0.25 | 0.37 | rose-pink | orange yellow turning |
| 6-Methylspinaceamine | 0.51 | 0.56 | emerald green | into orange red |