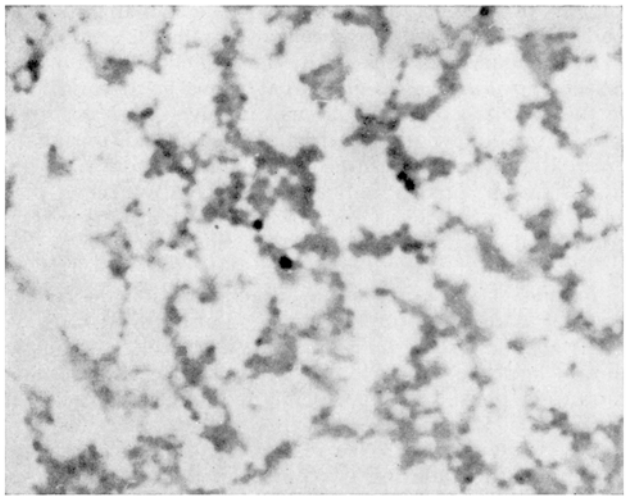

Staphylococcus pyogenes var. *aureus*, showing 'partially acid-fast' positive (dark) and 'partially acid-fast' negative (light) cells. On the right, 'partially acid-fast' positive cells just after division. Approx. 1600 ×.

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.

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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.

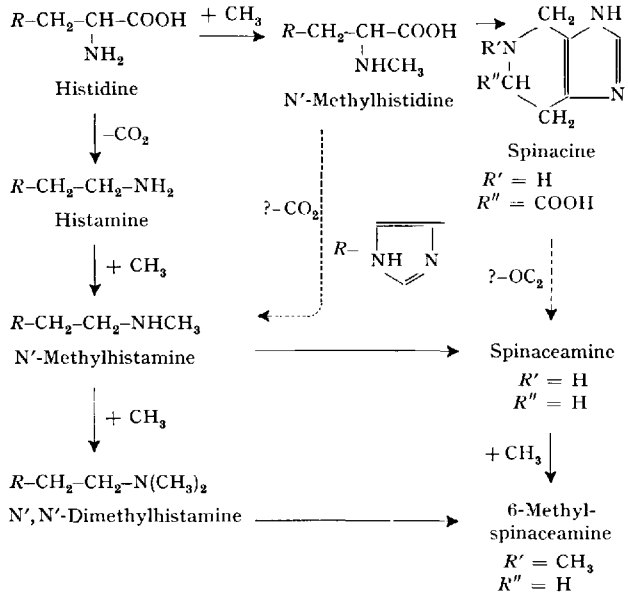
The accompanying Table shows the Rf values of the above amines with two different solvent systems and the colour reactions produced on paper by the Pauly reagent and the Folin reagent for aminoacids.

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

The approximate content in imidazoalkylamines of the dry skin obtained from 5 specimens of *Leptodactylus pentadactylus labyrinthicus* captured in Misiones in September 1961 was as follows (in μg of free bases per g dry tissue): histamine 360–400, N'-methylhistamine 250–300, N',N'-dimethylhistamine 100–120, spinaceamine 60–70, 6-methylspinaceamine 200–220.



The possible biochemical correlations among the different imidazolealkylamines of the *Leptodactylus* skin are illustrated below.

Details on methods and data obtained in other *Leptodactylus pentadactylus* species as well as in other amphibians will be presented in the paper *in extenso*, together with a full discussion of results.

Riassunto. Estratti di pelle di *Leptodactylus pentadactylus labyrinthicus* contengono elevati quantitativi di imidazolalchilamine, fra cui due derivati imidazo-*c*-piridinici finora ignoti in natura: la spinaceamina e la 6-metilspinaceamina.

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February 10, 1963.

The Sodium and Glucose Transports by *in vitro* Intestinal Preparations[1]

The experimental data reported here have been obtained on isolated everted sacs of small intestine of albino rat prepared according to Wilson and Wiseman[2]. As incubating mucosal fluid, the Krebs–Henseleit[3] solution or a modified one (see Tables) was used. The intestinal sacs were incubated for 1 h and 30 min at 38°C or at 28°C; a low temperature seems to improve the efficiency of the preparation. The volume of the serosal fluid at the end of the incubation period was determined by weighing. The fresh weight of the emptied intestinal sac was also determined at the end of the experiment.

The glucose concentrations in the mucosal and serosal fluids were determined at the end of the experiment according to the method of King[4]; from these concentrations, the glucose which disappears in the mucosal fluid, and the glucose which appears in the serosal fluid, were calculated. The sodium concentration in the serosal fluid was determined at the end of the experiment by means of an 'Optica CF 4' flame spectrophotometer (λ = 589 mμ); from this concentration the sodium gain of the serosal medium (net sodium transfer) was calculated. The lactic acid concentrations in the mucosal and serosal fluids were determined at the end of the experiment according to the method of Barker and Summerson[5]; from these concentrations the whole production of lactic acid was calculated. The glucose and lactic acid determinations allow us to define the following glucose quantities: glucose absorption = glucose disappearing in the mucosal fluid; glucose transfer = glucose appearing in the serosal fluid; glucose utilization = glucose absorption minus glucose transfer; unrecovered glucose = absorbed glucose minus transferred glucose minus glucose appearing as lactic acid.

In a previous report Rossi et al.[6] emphasize the fact that the utilization of glucose by the isolated perfused intestine, according to the technique of Smyth and Taylor[7], seems not to be dependent on the sodium concentration in the mucosal fluid, when NaCl is replaced by isosmolar quantities of urea or mannitol; on the contrary, the glucose transfer seems to be correlated with the net sodium transfer and with the sodium concentration in the mucosal fluid.

Present observations substantially confirm the previous ones. If the values of net sodium transfer obtained in control experiments are plotted against the values of glucose absorption (Figure, A) or of glucose transfer (Figure, B), a correlation appears in the second case. A correlation between net sodium transfer and unrecovered glucose cannot even be detected. In the experiments in which 2,4-dinitrophenol (DNP) was used (Table I), in spite of the marked inhibition of the sodium pump, there is little change in glucose absorption, in accordance with similar observations of Matthews and Smyth[8] and Lippe et al.[9], and the glucose transfer is completely abolished. Significant differences in the production of lactic acid do not appear under these experimental conditions, so that the change in glucose absorption may be simply

[1] This work has been supported by a research grant of the Consiglio Nazionale delle Ricerche, Roma.
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