Rev. Biol. Trop., 7 (2): 133-141, 1959

Decarboxylation Pathways of Amino Acids in Some Mammal and Bird Tissues*

by

John Claudatus** Pedro Gutierrez**
Donald Lefemine**

(Received for publication August 7, 1959)

In a previous study (1) we have described some observations on the decarboxylation of amino acids in fish tissues.

The present paper reports the results obtained using the same paper chromatographic technique (2, 3) with tissue homogenates of some terrestrial animals.

These findings do not represent a systematic study of the decarboxylation pathway of amino acids of the terrestrial animals because they were aimed to give collateral information on the metabolism of indolalkylamines and histamine but they add some data in the very little explored field of Comparative Biochemistry.

MATERIAL AND METHODS

The tissues of laboratory animals were used immediately and those obtained from the slaughter house were immediately frozen and used after 2-3 days.

It has been shown for fish tissues (1) that freezing for weeks does not change the ninhydrin patterns of amino compounds.

The homogenates were made with cold M/15 phosphate buffer pH 7.5 (1:2) and strained through cheese cloth.

For the tissue blanks 0.5 ml of homogenate, 0.4 ml pyridoxal phosphate (0.1 mg/ml) and 1.2 ml phosphate buffer pH 7.5 were added. The samples containing serotonin or histamine respectively were obtained by adding to 0.4

* Supported by a research grant from the National Cancer Institute (C3307) Department of Health, Education and Welfare.

** The Cancer Institute at Miami, Miami, Florida.

ml homogenate, 0.4 ml pyridoxal phosphate, 0.4 ml M/50 of the corresponding amine and 0.8 ml phosphate buffer pH 7.5. Amine blanks were prepared by mixing 0.4 ml M/50 amine with 0.4 ml pyridoxal phosphate (0.1 mg/ml) and 1.2 ml buffer.

For the samples incubated under N_2 and O_2, incubation time was 2 hours at 37ºC in a shaking incubator. All samples, incubated and non-incubated, were added with 5 ml ethanol/acetic acid (9/1), centrifuged, and 2 ml of the supernatant were evaporated in vacuum over NaOH pellets. The residue was picked up in 1 ml ethanol (95%) and centrifuged; 0.25 ml of clear supernatant was spotted, under an atmosphere of N_2 on Whatman Nº 1 chromatographic strips 1 x 20 in.; evaporation was hastened by infrared heat. The strips were developed by descending chromatography, in a chromatocab, using propanol water 5/1 as a solvent (7). After drying, the chromatograms were sprayed with 0.25% ninhydrin in butanol and heated in an oven for 15 minutes at 65ºC (6).

RESULTS

Pig liver and kidney

As can be seen from Fig. 1, the pig kidney has three lower bands corresponding to three preformed amines (group of three strips Nº 4); strong disappearance of serotonin is observed under anaerobic incubation of serotonin in kidney but not in liver (second strip group Nº 5); probably by conjugation; both liver and kidney show disappearance of serotonin after incubation under 0_2 (third strip group Nº 2 and Nº 5); no significant disappearance of histamine was observed in kidney or liver (group Nº 3 and Nº 6) indicating very little or absence of histaminase. The thin bands observed over the bands of histamine in liver samples (group Nº 3) show the presence of free glucose (4) which is absent in kidney homogenate. There is a difference in the nature of one of the five free amino acids between pig liver and kidney, three being identical as can be seen from five bands of all the groups.

Ox adrenals

As can be seen from Fig. 2 (group of strips Nº 1) there are present in ox adrenals two preformed amine-like compounds (the two lower bands); these two compounds are not dopa-amine or noradrenalin because under the present experimental conditions the above mentioned known amines would have different Rfs and tan color. The observed unknown bands are blue. The nature of these compounds is under study. As can be seen from the groups of strips Nº 3 and Nº 4, added serotonin or histamine are not appreciably metabolized by ox adrenal homogenate indicating the absence of both mono and diamine oxidase or conjugases.

GUINEA PIG LIVER

Fig 3 shows that in the guinea pig liver serotonin is strongly metabolized anaerobically and aerobically (group Nº 6).

Tarauco corythaix LIVER

Fig. 4 shows strong anaerobic and aerobic catabolism of added serotonin (strong conjugase? and monoamine oxidase activities), and very slight if any transformation of histamine (no conjugases or histaminase).

Cathartes aura septentrionalis (TURKEY BUZZARD) LIVER

Fig. 5 shows absence of anaerobic metabolism of serotonin and histamine (absence of conjugases? and strong aerobic metabolism (monoamine oxidase and diamine oxidase activity). It is well known (5) that the turkey buzzard liver has great histaminase activity.

ACKNOWLEDGEMENT

We would like to acknowledge the interest and encouragement given to us by Dr. J. E. Ayre, Director of the Cancer Institute at Miami; and to thank Miss M. D. Bellomy for the efforts extended in procuring a specimen of *Tarauco corythaix;* also, Dr. R. H. Smithers, Director of the National Museum, Bulawayo, Southern Rhodesia, for callecting the specimen; also, Pan American World Airways, for all transportation arrangements; and Dr. L. Mitchell, for supplying ox adrenals used in our investigations.

SUMMARY

The following data were obtained on the decarboxylation pathways of amino acids in some mammal and bird tissue homogenates:

1. PIG KIDNEY AND LIVER: Three preformed amine-like compounds were observed in the kidney and strong disappearance of added serotonin during anaerobic and aerobic incubation (strong conjugases? and monoamine oxidase activities). No significant disappearance of added histamine was found after anaerobic or aerobic incubation.

In the liver serotonin disappeared only after aerobic incubation. Free glucose (reacting with the added amines) was present in the liver but not in the kidney.

2. OX ADRENALS: Two preformed amine-like compounds were present which are not noradrenalin or dopa amine. Absence of conjugases or amine oxidases.

3. GUINEA PIG LIVER: Strong disappearance of added serotonin after

anaerobic incubation. No significant disappearance of added histamine in both types of incubations.

4. *Tarauco corythaix* LIVER: Strong anaerobic and aerobic disappearance of added serotonin and very slight if any disappearance of histamine in both types of incubation.

5. *Cathartes aura septentrionalis* (TURKEY BUZZARD) LIVER: Absence of anaerobic metabolism of added serotonin or histamine (absence of conjugases?) and strong aerobic disappearance of both amines after incubation with both amines, (strong mono and diamine oxidase activities).

RESUMEN

Los autores obtuvieron los siguientes resultados sobre la decarboxilación de aminoácidos en algunos tejidos homogenizados de mamíferos y aves.

1. RIÑÓN E HÍGADO DE CERDO: Fueron encontradas tres aminas preformadas y fuerte desaparición de la serotonina añadida, fuese por incubación aeróbica o anaeróbica (fuertes conjugasas? y actividad de monoamino oxidasas).

Después de incubación aeróbica y anaeróbica no se apreció desaparición de la histamina añadida. En el hígado la serotonina desapareció solamente después de la incubación aeróbica. Se encontró glucosa libre (que reacciona con las aminas añadidas) en el hígado, pero no en el riñón.

2. SUPRARRENALES DE BUEY: Se encontraron dos componentes que parecen ser aminas y que no son noradrenalina ni dopamina. Hay ausencia de conjugasas y amino oxidasas.

3. HÍGADO DE COBAYO: Se observó fuerte desaparición de la serotonina añadida en incubaciones aeróbicas y anaeróbicas, al mismo tiempo que no se observó desaparición significativa de la histamina añadida en ninguna de las incubaciones.

4. HÍGADO DE *Tarauco corythaix*: Durante las incubaciones aeróbicas y anaeróbicas se observó fuerte desaparición de la serotonina añadida y muy leve, si no ninguna, desaparición de la histamina.

5. HÍGADO DE *Cathartes aura septentrionalis:* Se observó ausencia de metabolismo anaeróbico de serotonina y histamina añadidas (ausencia de conjugasas?) y marcada desaparición aeróbica de las dos aminas después de la incubación (fuerte actividad de mono y diamino oxidasas).

REFERENCES

1. CLAUDATUS, J., P. GUTIERREZ & D. LEFEMINE
Decarboxylation pathways of amino acids in fish tissues. *Bull. Mar. Sci. Gulf & Carib.* (in press).

2. CLAUDATUS, J., & S. GINORI
1957. Blocked kynurenine pathway of tryptophan metabolism in hepatoma. *Science* **125:394.**

3. CLAUDATUS, J., P. GUTIERREZ & D. LEFEMINE
1957. Absence of a liver factor reacting with indolalkylamines in hepatoma. *Arch. Biochem. & Biophys.* 72:316.

4. CLAUDATUS, J., P. GUTIERREZ & D. LEFEMINE
The ninhydrin colored compounds formed by reacting aldoses with histamine and indolalkylamines. (In preparation).

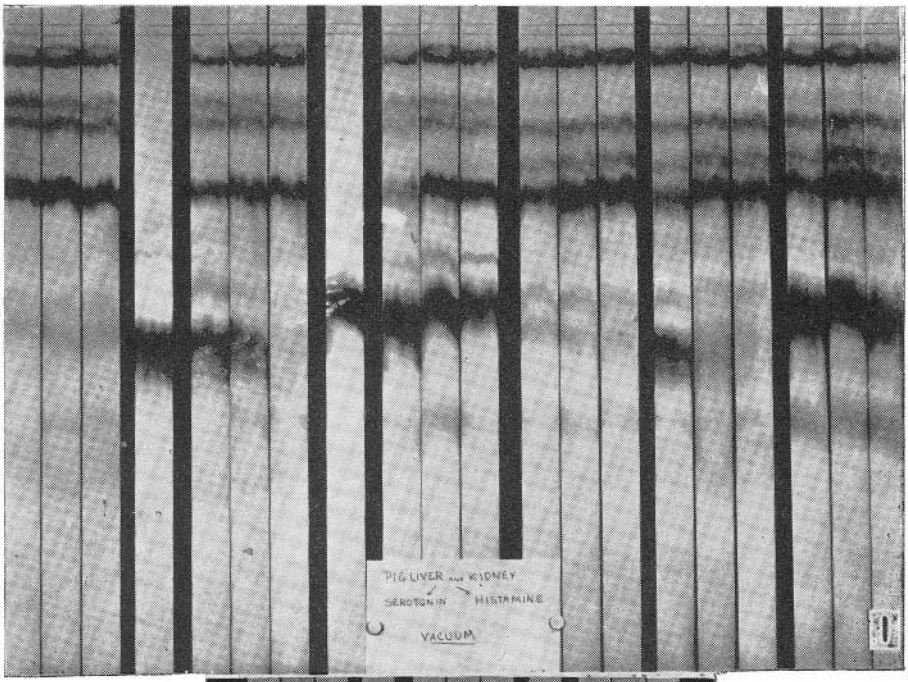
5. EUSTIS, A. C.
1915. *Biochem. Bull.* N. Y. 4-97.

6. KAY, R. E., D. C. DENIS & C. ENTERMAN
1956. Quantification of the ninhydrin color reaction as applied to paper chromatography. *Arch. Biochem. & Biophys.* 63:141.

7. UDENFRIEND, S., E. TITUS & J. WEISBACH
1955. The estimation of 5HO tryptamine (serotonin) in biological fluids. *J. Biol. Chem.* 216:499.

Fig. 1: *Metabolism of serotonin and histamine in pig liver homogenates.* In each group of three strips the first strips is non-incubated, the second N_2 and the third 0_2 incubated for 2 hours at 37°C. The single strips of amines blank are non-incubated (no change was observed after incubation of under N_2 or 0_2). The first group of three strips are pig liver homogenate blanks. The first single strip is serotonin blank. The second group of three strips has samples added with serotonin. The second single strip is histamine. The third group of three strips has liver homogenate samples added with histamine. The fourth group of three strips has pig kidney homogenate blanks. The fifth group pig kidney added with serotonin and the sixth group kidney homogenate added with histamine.

Fig. 2: *Metabolism of serotonin and histamine in ox adrenals homogenates.* In each group of three strips the first is non-incubated, the second N_2 incubated and the third 0_2 incubated. The first group of three strips has the adrenal homogenate blanks. The first single strip is the serotonin blank non-incubated; the second group has the homogenate added with serotonin. The second single strip is the histamine blank; the third group has the homogenate added with histamine.


PIG LIVER and KIDNEY
SEROTONIN
HISTAMINE
VACUUM
1

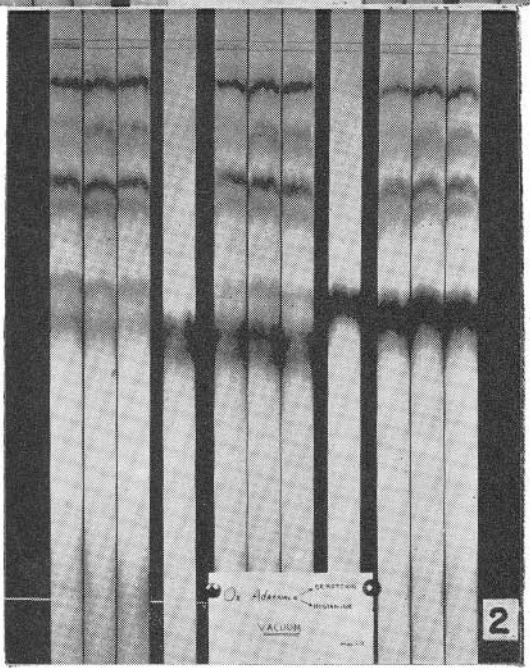
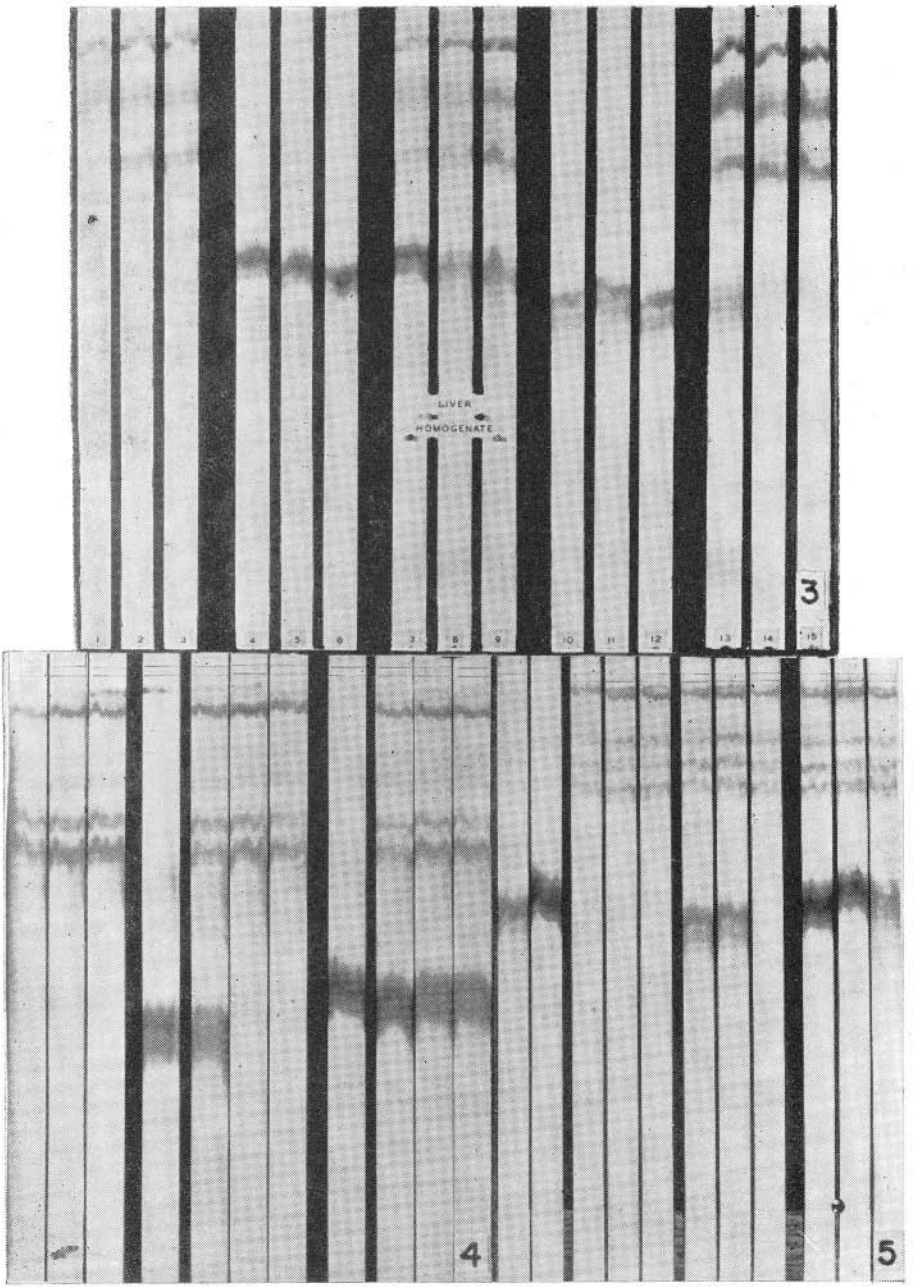

Ox Adrenal
VACUUM
2

Fig. 3: *Metabolism of serotonin and histamine in guinea pig liver homogenates.* In each group of three strips the first strip is non-incubated, the second N_2 and the third 0_2 incubated. The first group has the guinea pig liver homogenate blanks; the second histamine blanks; the third liver homogenate added with histamine; the fourth serotonin blanks and the fifth homogenate added with serotonin.

Fig. 4: *Metabolism of serotonin and histamine in Tarauco liver homogenate.* In each group of three strips the first strip is non-incubated, the second N_2 and the third 0_2 incubated. The first group has the tauraco bird liver homogenate blanks; the first single strip is serotonin blank; the second group of strips has homogenate added with serotonin; the second single strip is histamine blank and the third group homogenate added with histamine.

Fig. 5: *Metabolism of serotonin and histamine in turkey buzzard liver homogenates.* The first strip is serotonin blank; the second histamine blank. In the following group, of three strips the first strip is non-incubated, the second N_2 and the third O_2 incubated. The first group of three strips has liver blanks, in the second the homogenate is added with serotonin and in the third group is added with histamine.


LIVER
HOMOGENATE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
3
4
5