STUDIES OF CITRIC ACID METABOLISM IN HEART MUSCLE

by

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INTRODUCTION

Our present knowledge in the metabolism of citric acid *in vitro* in animal tissues is derived almost entirely either from experiments with tissue preparations possessing both synthesizing and utilizing properties regarding this acid, or from experiments carried out by means of crude preparations of one or other of the enzyme systems involved in citric acid metabolism, therefore without dynamic connections with other metabolic pathways leading to the formation of citric acid.

The aim of the experiments summarized in this paper has been to obtain heart muscle preparations which favour the formation of citric acid but do not utilize citric acid, so that it could accumulate in quantities sufficient to be estimated.

Although some intermediate steps are still rather obscure, starting with pyruvic acid as the principal precursor of citric acid it is possible to consider two pathways of formation:

(I) "Tricarboxylic acid" pathway: Pyruvate → oxaloacetate $\xrightarrow{+\text{ pyruvate}}$ oxalocitraconitate → (isocitrate ⇄ *cis*-aconitate ⇄ citrate). This mechanism established by WOOD and co-workers[1] is most consonant with the experiments done by EVANS AND SLOTIN[2] and those of WOOD and co-workers[3].

(II) "Citrogenase" pathway: Pyruvate → oxaloacetate $\xrightarrow{+\text{ acetoacetate}}$ citrate (or: isocitrate ⇄ *cis*-aconitate ⇄ citrate?). According to BREUSCH[4, 5] there exists a special enzyme called "citrogenase" which condenses acetoacetate with oxaloacetate to citric acid.

It has not yet been decided whether these two pathways exist independently of one another or not. It is also undecided whether the BREUSCH condensation of acetoacetate with oxaloacetate leads directly to citric acid formation or to another of the compounds of the aconitase equilibrium. Provisionally accepting the existence of both pathways we call them the "tricarboxylic acid pathway" and the "citrogenase pathway" of citric acid formation. For a discussion of this problem see STOTZ[6] and KREBS AND EGGLESTON[7].

In the present experiments an attempt has been made to study both these pathways, especially the citrogenase pathway, since its rôle in citric acid metabolism remains obscure. For this purpose two tissue preparations were made, one with intact "citrogenase" (suspension no. 2), and one in which citrogenase activity was removed by

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washing (suspension no. 3). Both suspensions contained aconitase and both on addition of boiled muscle extract produced citric acid but did not metabolize it further. On adding bicarbonate to suspension no. 2, an additional amount of citric acid was formed. This did not occur when bicarbonate was added to suspension no. 3. This suggests that suspension no. 2 contains an enzyme system leading to the formation of citric acid which is additional to the system present in suspension no. 3.

EXPERIMENTAL

MATERIAL AND METHODS

Reagents

Sodium pyruvate: product of "Hoffmann La Roche", Basle.
Citric acid, sodium bicarbonate, sodium hydroxide: analytical reagents.

Estimation of citric acid

The procedure adopted for the determination of citric acid is based upon the pentabromoacetone method. The substances interfering with the determination of citric acid were removed by boiling the acidified solution previous to treatment with KBr (BREUSCH[8]; see also KREBS AND EGGLESTON[7]).

The colorimetric determinations were made with the aid of the Pulfrich photometer. It is known that the maximum absorption of the colour developed from the test substance and the maximum transmittance of the filter used should be at the same wave length. This assures *ceteris paribus* maximum sensitivity of the photometric method.

With the pentabromoacetone method so far the colorimetric determinations have been made at different wave lengths. PUCHER, SHERMAN, AND VICKERY[9] used the Pulfrich photometer with a light filter no. S–43 (maximal transmission at 434 mμ), SPECK, MOULDER, AND EVANS[10] read the samples in an Evelyn Colorimeter with filter no. 420 (range of transmission 350–460 mμ with a maximum at 420 mμ). HUNTER AND LELOIR[11] used a Klett-Summerson photoelectric colorimeter with filter 42 (420 mμ and range of transmission 400–450 mμ). JOSEPHSON AND FORSSBERG[12] recommend determinations at 430 mμ. KALNITSKY[13] carried out determinations at 440 mμ with a Coleman Universal Spectrophotometer. NATELSON, LUGOVOY ,AND PINCUS[14] performed readings at 450 mμ.

Therefore a study was made of the absorption spectrum of the coloured substance involved in this determination by means of a Beckman photoelectric quartz spectrophotometer. Readings made between 15 and 30 min after colour development at 10° C give the peak at 446–448 mμ, which is generally in agreement with the results of NATELSON, LUGOVOY, AND PINCUS[14].

In the present experiments the colour was read by using filter S–45 of the Pulfrich photometer, as the standardization of the filters of the Pulfrich photometer carried out by NIEMEYER[15] gives the following values for S–45 and S–47:

wave length in Å	per cent transmittance	
	S–45	S–47
4400	5.25	1.85
4500	4.25	4.76
4600	1.35	4.95

Thus maximum sensitivity can be obtained with filter S–45.

The samples were deproteinized by means of trichloroacetic acid. The further steps of the determination were carried out as described by NATELSON, LUGOVOY, AND PINCUS[14]; with use of glass-stoppered test tubes with graduations at 2, 3, and 5 ml, colour development below 10° C, readings between 15 and 60 min after colour development. The best stabilization of the colour was obtained by means of this procedure. The solution was decolourized with the minimal amount of 15% hydrogen peroxide. *n*-Heptane, b.p. 96–97° was used as solvent. The colour is read against the solution of sodium sulphide as "blank" with filters S–45 and S–66 in a cell of 2½ cm length. Correction is made for cloudiness by subtracting the optical density obtained with S–66 from the optical density obtained with S–45. The measurement in a solution of pure citric acid carried out with the Beckman spectrophotometer showed that the absorption with S–66 should be nil. Using this technique and standardizing the conditions of the procedure (*e.g.*, shaking with the same speed: 200 oscillations per min, etc.) amounts of the order of 10 to 100 micrograms of citric acid can be determined with an accuracy of 5%.

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Tissue preparations

The tissue used was pigs heart, obtained from the slaughter house. As soon as possible after killing the animal the heart was removed, cooled to 0° C and maintained at this temperature until used.

The muscle of the left and right ventricles was minced in the Latapie mincer. The Latapie-mince of the left ventricle was used for preparation of suspensions no. 1, no. 2, and no. 3; the mince of the right ventricle was used for preparation of boiled muscle extract (b.m.e.).

Suspension no. 1: 10 g of Latapie-mince was suspended in 50 ml of 0.1 M phosphate buffer p_H 7.4.

Suspension no. 2: 10 g of Latapic-mince was suspended in 50 ml of distilled water then shaken for 10 min (200 oscillations per min).

The suspension was diluted to 75 ml with distilled water and centrifuged at 2500 r.p.m. for 30 min. The supernatant fluid was discarded and the sediment resuspended in 50 ml of distilled water, shaken for 10 min, diluted to 75 ml with distilled water and centrifuged for 30 min.

The supernatant fluid was discarded again and the procedure repeated once more. Finally the sediment was thoroughly resuspended in 50 ml of 0.1 M phosphate buffer p_H 7.4 and stored at 0° C.

Suspension no. 3: The third sediment resulting from the preparation of suspension no. 2 was suspended in 50 ml of 0.5% sodium bicarbonate, shaken for 10 min, diluted to 75 ml with 0.5% sodium bicarbonate, and centrifuged at 2500 r.p.m. for 15 min. The supernatant fluid was discarded and the procedure twice repeated. Finally the third sediment was resuspended in 50 ml of 0.1 M phosphate buffer p_H 7.4, and stored at 0° C.

Boiled muscle extract was prepared according to KREBS AND EGGLESTON[16] by mixing one part of fresh, finely minced muscle with one part of water, placing in a boiling water bath for 10 min and filtering.

The proportion of extract to total volume was usually 1:3. As a rule the boiled muscle extract was prepared at the same time as the corresponding suspensions. All steps of tissue preparations were carried out at 15°–18° C, except the mincing in the Latapie-mincer. This step was carried out at 0° C. The p_H of suspensions 2 and 3 was 7.4; the p_H of the boiled muscle extract 6.0 (measured with the aid of "Lyphan" indicator paper, which had been checked with the glass-electrode).

RESULTS

Suspension no. 1 freshly prepared or stored for various times (from 6 to 96 h) at 0° C contained from 0.27 to 0.44 micromoles of citric acid per g of tissue. PUCHER, SHERMAN AND VICKERY[9] found 0.076 micromoles per g of dog heart muscle and HALLMAN[17] only 0.019 micromoles per g of bull heart muscle. In my boiled muscle extracts I found a content of the same order of magnitude as the figure given by HALLMAN, *viz.* 0.013 micromoles per g of tissue. Since suspension no. 1 contained much more citric acid than the muscle from which it was prepared, it follows that citric acid is rapidly formed from precursors present in the tissue.

Samples of suspension 1 which had been stored for 24 to 72 h at 0° C and had then been shaken aerobically in a bath at 37° for 1 hour contained from 2.04 (72 h at 0° C) to 4.80 (24 h at 0° C) micromoles of citric acid per g of tissue.

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Hence under these conditions (*i.e.*: 8 1/3 g of minced tissue suspended in total volume of 50 ml; 0.083 M phosphate buffer, p_H 7.4) citric acid is formed more rapidly than it is metabolized.

With $3^1/_3$ g of tissue suspended in total volume of 50 ml, 0.033 M phosphate buffer, p_H 7.4—on the contrary—the rates of formation and utilization of citric acid seem to be equal (Table I).

TABLE I

EFFECT OF PROPORTION OF TISSUE TO MEDIUM AND OF MOLAR CONCENTRATION OF PHOSPHATE BUFFER ON CITRIC ACID FORMATION IN PIG HEART MUSCLE (AEROBIC)

a

$8^1/_3$ g of minced tissue suspended in a total volume of 50 ml; 0.083 M phosphate buffer, p_H 7.4; shaken at 38° C in air. Tissue examined after storage, as susp. No. 1, at 0° C during 72 hours. Heart No. 13.

Minutes incubated	0	10	20	30	40	50	60	70
μmoles of citric acid formed per g of wet tissue	0.35	0.77	1.29	1.69	2.05	2.39	2.53	2.62

b

$3^1/_3$ g of minced tissue suspended in a total volume of 50 ml; 0.033 M phosphate buffer, p_H 7.4; shaken at 38° C in air. Tissue examined after storage as susp. No. 1 at 0° C during 6 hours. Initial p_H of the incubate: 7.2; final p_H: 7.2. Heart No. 10.

Minutes incubated	0	10	20	30	40	50	60	70	80
μmoles of citric acid formed per g of wet tissue	0.33	0.31	0.14	0.10	0.06	0.21	0.26	0.28	0.33

Indeed, as is shown in Tables II and III citric acid added to the latter suspension disappears at about the same rate as it is formed from added pyruvate.

These experiments indicate the importance of the concentration of the tissue particles and of the composition of the medium in the balance between citric acid formation and utilization. These experiments show also that neither suspension no. 1 undiluted, nor diluted can be used for studying the formation of citric acid undisturbed by simultaneous disappearance of citric acid.

TABLE II

AEROBIC FORMATION OF CITRIC ACID FROM PYRUVATE IN MINCED PIG HEART MUSCLE

$3^1/_3$ g of minced tissue suspended in a total volume of 50 ml; 0.033 M phosphate buffer, p_H 7.4; 218 μmoles of sodium pyruvate added per g of wet muscle. Incubated as usual at 38° C in air. At intervals 5 ml of the suspension were removed for analysis and the reaction was stopped by addition of trichloroacetic acid. The tissue was examined after storage as suspension No. 1 at 0° C during 24 hours.

Minutes incubated	0	10	20	30	40	50	60	70
μmoles of citric acid per g wet tissue	0.37	0.80	1.17	1.57	2.04	2.51	2.86	3.00

TABLE III

CITRIC ACID UTILIZATION IN MINCED PIG HEART MUSCLE (AEROBIC)

$3^1/_3$ g of minced tissue suspended in a total volume of 50 ml; 0.033 M phosphate buffer, p_H 7.4; 10.4 μmoles of citric acid added. Incubated at 38° C as usual in air. At intervals, 5 ml of the suspension were removed for analysis and the reaction was stopped by addition of trichloroacetic acid. The tissue was examined after storage as suspension No. 1, at 0° C during 6 hours. Heart No. 10.

Minutes incubated	0	10	20	30	40	50	60	70	80
μmoles of citric acid utilized (—) per g of wet tissue	+0.03	—1.97	—2.65	—2.93	—3.05	—3.14	—3.05	—3.05	—3.02

This difficulty was overcome by using suspensions 2 and 3 prepared at the same concentration of tissue and phosphate buffer of p_H 7.4 as diluted suspension no. 1, and restored with boiled muscle extract.

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These suspensions do not contain any citric acid, but it is rapidly formed after adding pyruvate, while added citric acid is not metabolized. Tables IV and V give examples of these experiments.

TABLE IV

COMPARISON OF CITRIC ACID UTILIZATION IN RESTORED SUSPENSIONS NO. 2 AND 3 (AEROBIC)

10 ml of boiled muscle extract; quantity of suspension No. 2 or of No. 3 corresponding to $3^1/_3$ g of wet tissue in a total volume of 50 ml; 0.033 M phosphate buffer, pM 7.4; 0.52 μmoles of citric acid added. Incubated at 38° C as usual in air. At intervals, 5 ml of the suspensions were removed for analysis and the reaction was stopped by addition of trichloroacetic acid. The suspensions were examined after storage at 0° C during 48 hours. Heart No. 11.

Minutes incubated	0	10	20	30	40	50	60	70
μmoles of citric acid per g of wet tissue (1.56 μmoles per g added) in suspension 2	1.71	2.06	2.34	2.62	2.62	2.64	2.62	2.62
in suspension 3	1.76	2.25	2.34	2.36	2.39	2.53	2.67	2.67

TABLE V

RATE OF CITRIC ACID FORMATION IN RESTORED SUSPENSION NO. 2 FROM SODIUM PYRUVATE AND PRECURSORS PRESENT IN B.M.E. (AEROBIC)

10 ml of boiled muscle extract; quantity of suspension No. 2 corresponding to $3^1/_3$ g of wet tissue in a total volume of 50 ml; 0.033 M phosphate buffer, pH 7.4; 218 μmoles of sodium pyruvate per g of wet muscle. Incubated as usual at 38° C in air. At intervals, 5 ml of the suspension were removed for analysis. The suspension was examined after storage at 0° C during 48 hours. Heart No. 8.

Minutes incubated	0	10	20	30	40	50	60	70	80
μmoles of citric acid formed per g of wet tissue	0.37	2.72	3.79	4.73	5.15	5.34	5.71	5.71	5.57

It may be seen from Table IV (citric acid added; no pyruvate added) that the amount of citric acid not only does not diminish, but even increases a little. This increase is due to the formation from precursors present in the boiled muscle extract.

The rates of the formation of citric acid from precursors with suspensions no. 2 and no. 3 are the same, which may be taken as evidence supporting the view that there are no cardinal differences in metabolism of citric acid by suspension no. 2 and no. 3, except for citrogenase activity in suspension no. 2.

Suspensions 2 and 3 prepared from various pig hearts show differences in activity, by which is meant the amount of citric acid produced in incubation experiments. This seems to be due to unequal properties of boiled muscle extracts used in different experiments.

As Table VI shows the amount of citric acid formed in anaerobic conditions is small, even in the presence of added pyruvate. This confirms WEIL-MALHERBE[18], who found no anaerobic formation of citric acid from oxaloacetic acid in tissue slices and WIELAND AND ROSENTHAL[19], who reported that oxygen was necessary for the formation of citric acid from oxaloacetic acid and acetoacetic acid by kidney tissue.

TABLE VI

ANAEROBIC FORMATION OF CITRIC ACID FROM PYRUVATE IN MINCED PIG HEART MUSCLE

$3^1/_3$ g of minced tissue suspended in total volume of 50 ml; 0.033 M phosphate buffer, pH 7.4; 218 μmoles of sodium pyruvate per g of wet muscle. Samples of this suspension were placed in Thunberg tubes. These were evacuated three times and filled three times with nitrogen purified by passage through an alkaline solution of pyrogallol. Incubated at 38° C as usual. At intervals, Thunberg tubes were taken for analysis. The reaction was stopped by addition of trichloroacetic acid. The tissue was examined after storage, as suspension No. 1, at 0° C, during 24 hours. Heart No. 11.

Minutes incubated	0	30	60
μmoles of citric acid per g of wet tissue	0.37	0.54	0.51

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TABLE VII

EFFECT OF SODIUM BICARBONATE ON CITRIC ACID FORMATION IN RESTORED SUSPENSION NO. 2 AND NO. 3 FROM SODIUM PYRUVATE AND PRECURSORS PRESENT IN B.M.E. (AEROBIC)

2 ml of boiled muscle extract; quantity of suspension No. 2 or of No. 3 corresponding to $^2/_3$ g of wet tissue in a total volume of 10 ml; 0.033 M phosphate buffer, pH 7.4; 218 μmoles of sodium pyruvate per g of wet muscle; quantities of sodium bicarbonate, added in the form of 1% solution, specified below. Incubated as usual at 38° C in air. Period of incubation 60 min. pH was determined by means of "Oxyphen" indicator paper. Suspensions examined after storage at 0° C during 24 hours. Heart No. 8.

μmoles of $NaHCO_3$ per g of wet tissue	μmoles of citric acid formed per g of wet tissue; pH before and after incubation			
	Suspension No. 2		Suspension No. 3	
	μmoles	pH	μmoles	pH
0	5.83	7.2–7.2	5.80	7.2–7.2
45	6.93	7.2–7.2	5.97	7.2–7.2
89	9.74	7.2–7.4	5.83	7.2–7.4
134	8.71	7.2–7.4	4.92	7.2–7.4
179	9.27	7.2–7.5	4.50	7.2–7.9
223	10.26	7.2–7.6		
268	9.27	7.2–7.9		
357	7.87	7.2–8.1	4.29	7.2–8.1

FISHER's t-test for the difference between the bicarbonate effect in suspensions No. 2 and No. 3 equals 4.86. P = 0.001 (highly significant).

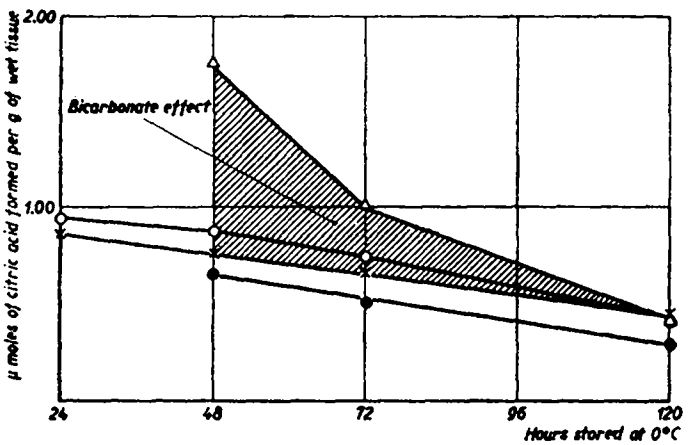

Fig. 1. *Aerobic formation of citric acid in restored suspension No. 2 and 3 from sodium pyruvate and precursors present in b.m.e.* 10 ml of boiled muscle extract; quantity of suspensions corresponding to $3^1/_2$ g of wet tissue in total volume of 50 ml; 0.033 M phosphate buffer, pH 7.4; 218 μmoles of sodium pyruvate per g of wet muscle. Incubated as usual, at 38° C, in air. Heart No. 6. Period of incubation: 60 min. ×——× suspension 2 without bicarbonate; o——o suspension 3 without bicarbonate; △——△ suspension 2 with 179 μmoles of bicarbonate added per g of wet tissue; •——• suspension 3 with 179 μmoles of bicarbonate added per g of wet tissue.

Table VII shows an example of the effect of adding various amounts of sodium bicarbonate to suspension 2 and 3 (stored at 0° C for 24 h in the presence of added pyruvate). Sodium bicarbonate increases the production of citric acid with suspension 2 but not with suspension 3. The presence of the bicarbonate effect in suspension 2 is still detectable after 72 h of storage of this suspension (see Fig. 1). The stability of this effect is much greater than the stability of known citrogenase preparations (BREUSCH[4, 5]).

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DISCUSSION

I. Suspensions 2 and 3 do not appear to utilize citric acid, although they form it.

This may be explained by the observation of ADLER, EULER, GÜNTHER AND PLASS[20], according to which the isocitric dehydrogenase is removed from the minced tissue by washing with water.

In my suspensions the formation of oxaloacetate has to precede the formation of citric acid. It cannot be present before incubation with pyruvate, because according to SEI KANEKO[21] it is readily decarboxylated at room temperature. As malic dehydrogenase is also removed by washing with water (STRAUB[22]; GREEN[23]), the formation of oxaloacetic acid in both suspensions No. 2 and 3 must be due either to CO_2 fixation, or to a transamination reaction. The latter seems to be a theoretical possibility only.

There is no direct proof of fixation of CO_2 in citrate with animals. Neither isocitrate nor citrate has been isolated in tracer experiments with pigeon liver, in which CO_2 "tagged" with isotope C was used (WOOD[24]). The only existing proof of fixation of CO_2 in citrate is carried out as yet by FOSTER and coworkers[25] with *Aspergillus niger*. Tissue preparations described as suspension No. 2 and 3 seem to present possibilities for tracer experiments to demonstrate by direct proof CO_2 fixation in citrate resp. isocitrate in heart muscle and, may be, other tissues.

In my experiments tentative evidence—though also in an indirect way—concerning CO_2 fixation by heart muscle is obtained from the experiments with added sodium bicarbonate. These prove clearly that the supply of CO_2 is the limiting factor in citric acid formation.

II. In experiments with suspension No. 2 the formation of citric acid can be increased on adding bicarbonate. In experiments with suspension No. 3 this cannot be achieved.

Before attempting to explain this fact, it is necessary to consider again the difference between suspensions No. 2 and 3. The suspension No. 3 is prepared as for suspension No. 2 minus BREUSCH's "citrogenase preparation", which is removed by the bicarbonate extraction. The steps involved in the preparation of suspension No. 3 are based on BREUSCH's procedure for preparing citrogenase completed by observations of HUNTER AND LELOIR[11]. BREUSCH claims that his "citrogenase preparation" uses oxaloacetate and acetoacetate in the condensation reaction leading directly to citric acid.

The absence of bicarbonate effect in experiments with suspension No. 3 seems to support the view that the citrogenase pathway is the main pathway in citrate formation in heart mince.

III. The restoring effect of boiled muscle extract requires further investigation.

IV. As a medium for all incubation experiments phosphate buffer p_H 7.4 was used. The importance of the ionic composition of the medium is well known from the work of THUNBERG[33], MEYERHOF[34], HOLCK[35], AND GRÉVILLE[36]. KREBS AND EGGLESTON[16] found that the best suitable saline medium for the respiration of minced pigeon breast muscle is 0.1 M phosphate buffer, p_H 6.8–7.4. Other media used by different authors in studies upon citric acid metabolism contain Ca^{++}, K^+, sodium bicarbonate or glycine. THUNBERG[33], however, stated that the metabolism of minced muscle is strongly inhibited by Ca^{++} even in "physiological" concentrations.

Disturbances of the equilibrium between the formation and the removal of oxaloacetic acid in muscle suspension observed by BANGA[37] by using Ringer's solution are probably also due to its Ca content.

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acetic in muscle suspension observed by BANGA[37] by using Ringer's solution are probably also due to its Ca content.

GRÉVILLE[38] stated that the addition of Ca^{++} and K^{+} in physiological salt solution concentrations to a suspension of minced muscle has an inhibitory effect on both the formation and the removal of oxaloacetic acid.

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SUMMARY

1. The pentabromoacetone method for the determination of citric acid was studied; a modification of the procedure of NATELSON, LUGOVOY, AND PINCUS was used.

2. Two tissue preparations were obtained. The first by washing with water, the second by washing with water and then with 0.5% sodium bicarbonate solution. In both suspensions citric acid is formed after adding boiled muscle extract, while added citrate is not utilized.

3. The formation of citric acid is increased when sodium bicarbonate is added to the suspension washed with water only. With the suspension washed with water and with sodium bicarbonate solution this effect is not observed; this seems to be due to the fact that in this suspension the elimination of the "citrogenase" pathway is achieved.

RÉSUMÉ

1. La méthode à la pentabromacétone pour la détermination de l'acide citrique a été étudiée; une modification du procédé de NATELSON, LUGOVOY ET PINCUS a été adoptée.

2. Deux préparations de tissus de cœur de porc ont été obtenues; la première par lavage à l'eau seulement, la seconde par lavage à l'eau, puis avec une solution de bicarbonate de sodium à 0.5%. Dans les deux suspensions, de l'acide citrique est formé après addition d'extrait de muscle de cœur bouilli, tandis que du citrate additionné n'est pas utilisé.

3. La formation d'acide citrique est augmentée lorsque du bicarbonate de sodium est ajouté à la suspension lavée à l'eau seule. Avec la suspension lavée à l'eau et au bicarbonate, cet effet ne s'observe pas; la raison en est, semble-t-il, que l'élimination du citrogénase a été réalisée.

ZUSAMMENFASSUNG

1. Die Pentabromaceton-Methode zur Bestimmung von Citronensäure wurde untersucht; eine Abwandlung des Verfahrens von NATELSON, LUGOVOY UND PINCUS ist angewendet worden.

2. Zwei Schweineherzgewebe-Präparate wurden erhalten; das erste durch Waschen nur mit Wasser, das zweite durch Waschen mit Wasser und dann mit 0.5%-iger Natriumbicarbonat-Lösung. In beiden Aufschlämmungen wird Citronensäure nach Zufügung von gekochtem Herzmuskelextrakt gebildet, während zugegebenes Citrat nicht verwendet wird.

3. Die Bildung von Citronensäure wird vermehrt, wenn Natriumbicarbonat zu der nur mit Wasser gewaschenen Aufschlämmung zugegeben wird. Diese Einwirkung wird dagegen nicht beobachtet bei der mit Wasser und Bicarbonat gewaschenen Aufschlämmung; dies scheint davon herzurühren, dass die Eliminierung der Citrogenase erreicht worden ist.

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