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OLD AND NEW OBSERVATIONS ON THE CHEMICAL COMPOSITION OF *TRYPANOSOMA CRUZI* (1)

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SUMMARY

Available data are assembled by the author on the chemical constitution of culture form of *Trypanosoma cruzi.*

A few new data on the composition of its lipids are included. The lipid content is rather high, averaging 20% of dry substance.

A small amount of the insaponifiable material consists of cholesterol and a substance with ultraviolet absorption spectrum characteristic of a 5.7 diene.

Thus far, a few data on the fatty acids composition showed a small amount of C 8 to C 12 fatty acids and a high content of insaturated fatty acids, among which a C 15 acid was found.

Carbohydrate, nucleic acids and amino acids data were also reviewed.

Editor's summary

INTRODUCTION

Considerable information is available concerning the metabolism of *Trypanosoma cruzi* and other species of trypanosomes (review of literature in VON BRAND [2]). In contrast, relatively little is known about their chemical composition. This is not surprising because on the one hand their high rate of metabolism and the current interest in the dynamic phases of comparative biochemistry combine to make the trypanosomes excellent objects for metabolic studies. On the other hand, their small size renders it difficult to secure sufficient material for analytical studies dealing with their chemical composition. It seems nevertheless desirable to carry out such studies since they have many important implications. As a basis for elucidating the endogenous metabolism, for instance, it is essential to know whether an organism stores significant amounts of reserve substances and if it does, of what type they are. For immunological investigations a knowledge of the chemical nature of the antigenic substances is of obvious importance. Further-

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(1) Dedicated to the memory of Dr. Gaspar Vianna.

more, questions concerning the nature of the nucleic acids are directly relevant to endeavors to explain the mode of action of certain drugs (e.g. MORAES *et al.*[16]) and may be an important link in laying the foundation for a rational approach to chemotherapy.

An attempt is made in the present paper to assemble the available data on the chemical constitution of *Trypanosoma cruzi*. A few new data on the composition of its lipids are included. The presentation is by necessity restricted to the culture form since virtually no information on the chemical composition of the bloodstream form or the tissue stages is available.

GENERAL CHEMICAL COMPOSITION

The general chemical composition of the culture form of *Trypanosoma cruzi* grown on two types of media has been studied by VON BRAND *et al.*[3]. Their results are summarized in Table 1.

TABLE 1

General chemical composition of the culture form of *Trypanosoma cruzi* expressed as percent of fresh tissue.

	Flagellates grown on	
	Dialysate medium (Tobie and Rees, 1948)	Blood agar medium (Johnson, 1947)
Dry substance	15.5	16.4
Inorganic substances	2.42	Not determined
Polysaccharide	0.08	0.07
Total fermentable carbohydrates	0.17	Not determined
Lipids	3.12	2.80
Protein (N × 6.25)	8.23	6.96

It is evident that the flagellates harvested from both media gave, within reasonable limits of variation, identical results. It must, however, be stressed that the values of Table 1 represent only approximations. This is due to the fact that the protozoa required for the various analyses were collected by centrifugation and, before weighing, could not be freed completely from the Ringer's solution used to wash them.

The nature of the inorganic substances occurring in the bodies of the parasites has not been studied as yet. So far no reliable method of freeing them completely from the Ringer's solution mentioned above has been devised. Even a brief washing with distilled water leads at least to partial lysis and thus presumably to losses in inorganic substances.

CARBOHYDRATES

The culture form of *Trypanosoma cruzi* does not store appreciable amounts of low molecular weight carbohydrates in its body. FAIRBAIRN[7] found in its dry substance only 0.06 percent glucose and <0.05 percent trehalose, as compared to 3.1 and 0.8 percent respectively in the free-living ciliate *Tetrahymena pyriformis*. Small amounts of glucosamine have recently been described from hydrolyzates (WILLIAMSON & DESOWITZ[23]). The value for fermentable carbohydrates shown in Table 1 is based on fresh tissue and when recalculated on the basis of dry substance becomes appreciably higher than the sum of glucose and trehalose as given by FAIRBAIRN. In the case of total carbohydrate determinations the flagellates were first hydrolyzed with hydrochloric acid and the reducing power of the hydrolyzate was determined before and after digestion with yeast. Evidently this procedure leads to the determination of all fermentable carbohydrate, whether free in the body or cleaved from protein or lipid.

Some of the fermentable carbohydrate was probably also derived from an immunopolysaccharid occurring in the organisms. An active fraction had first been isolated by MUNIZ & FREITAS[17]; its chemical nature has subsequently been established by GONÇALVES & YAMAHA[12]. It is apparently a polysac-

charide-polypeptide complex containing 4.95 percent nitrogen, 0.62 percent phosphorus, and 42 percent carbohydrate calculated as glucose. The acid hydrolysate revealed eleven aminoacids and the following sugars: Glucose, glucosamine, xylose, mannose, and galactose in the molar ratio of 1:2:4:5:9.

In contrast to many other parasitic protozoa (review of literature in VON BRAND [1]), but in agreement with findings on other species of trypanosomes, the culture form of *Trypanosoma cruzi* does not store glycogen or other glucose polymers. It does contain small amounts of a polysaccharide (Table 1) which does not give an iodine color, has a specific rotation of $\alpha D + 28°$, and on acid hydrolysis yields galactose (VON BRAND *et al.*[3]). The sample studied was contaminated with nucleic acid derivatives and had been isolated with the help of strong KOH. Whether it was derived from the galactose — containing immunopolysaccharide mentioned above or whether it represents a special polysaccharide analogous to, but probably not identical with the galactogen stored by molluscs, must be clarified by further studies.

LIPIDS

The lipid content of *Trypanosoma cruzi* is rather high, averaging close to 20 percent of the dry substance (VON BRAND *et al.*[3]). It corresponds roughly to that reported for the bloodstream form of *Trypanosoma equiperdum* (IKEJIANI [13]).

Approximately half the lipids are acetone insoluble and this fraction contains 1.7 percent N and 3.6 percent P, indicating that it consists largely of phospholipids. Ten percent of the total lipids are represented by unsaponifiable material. At least a fourth of the unsaponifiable lipids consists of cholesterol which was characterized by melting point (145-147°), optical rotation ($\alpha D - 41°$), infrared absorption spectrum and analogous properties of the acetate and dibromide. In addition to cholesterol, a small amount of a substance with an ultraviolet absorption spectrum characteristic of a 5,7 diene was found, but not enough material for complete characterization was available.

The nature of the larger part of the unsaponifiable material remains to be elucidated; in the experiments reviewed here, it was recovered as resinous material. Further studies may well yield interesting results since in other invertebrates, various unsaponifiable substances have been found in addition to the sterols. Mention may be made here of the waxes, characteristic for the lipids of insects, the hydrocarbon heptacosan, found in the marine snail *Nassa obsoleta* (KIND *et al.*[15]), or the specific glycosides (ascarocides) recently described from parasitic nematodes (FOUQUEY *et al.*[11]).

The fatty acids of *Trypanosoma cruzi* have so far not been studied. Since the application of the newer techniques of lipid isolation, purification, and analysis seemed suited to this material, preliminary experiments in this direction were done in my laboratory. I am indebted to Mrs. J. E. Tobie for growing large numbers of trypanosomes on diphasic blood agar medium (JOHNSON [14]) and to Miss P. McMahon for extracting and purifying several grams of acetone-fixed trypanosomes according to a slight modification of the method of FOLCH *et al.*[10]. The samples were ground in a glass-Teflon homogenizer and the homogenate was filtered through sintered glass before being washed with 0.05 percent aqueous $CaCl_2$ solution. The extract was blown to dryness in a stream of nitrogen and the acetone-insoluble lipids were separated from the acetone-soluble fraction prior to the application of the purification-methylation procedure of STOFFEL *et al.*[21]. The resulting samples were sealed under nitrogen into glass vials and sent to Lachat Chemicals Inc., Chicago, Ill. for analysis. The samples, because of low yield of methyl esters resulting from the above procedure were re-esterified by the boron trifluoride-methanol method used in the Lachat Laboratories. The methyl esters were analyzed by Mr. K. Orvis of Lachat Chemicals by gas-liquid chromatography in an Aerograph Model A 90-C3, 4 wire detector, 6 foot 1/4 inch

stainless steel column containing 20 percent diethylene succinate polyester (Z100) coated on 60-80 mesh acid washed Chromosorb W. Identity of the peaks was determined by comparison with a mixture of known methyl esters of fatty acids and comparison of relative retention time with literature values. Further details of assay conditions are given in Table 2. The results of the analyses are shown in Figures 1 and 2 and Table 3. It is evident that both acetone-insoluble and acetone-soluble lipids contain a great variety of fatty acids. While both fractions appear to have many acids in common, the following main differences are apparent: The acetone-soluble lipids contain small, but significant amounts of C8 to C12 acids which were not seen in the acetone-insoluble fraction. Although a group of small peaks was present at the beginning of the latter's analysis (Fig. 1), it is probable that these do not represent the above acids, but are due to polymerization and degradation of polyunsaturated acids. The acetone-soluble contained a higher percentage of unsaturated acids (64.6 percent) than the acetone-insoluble fraction (43.5 percent). The latter, on the other hand, showed a much higher percentage (16.7 versus 1.0 percent) of an acid with the elution characteristics of a C15 acid. The latter finding, if substantiated by future chemical analysis, is rather interesting since the occurrence of large amounts of acids with uneven carbon numbers is rather rare in animal tissues, although the formation of C5 acids both by *Ascaris* (BUEDING & YALE [6]; BUEDING [5]) and the larvae of *Trichinella* (VON BRAND *et al.*[4]) is well known.

TABLE 2

Conditions of analysis of methyl esters by gas liquid chromatography.

	Acetone — insoluble fraction	Acetone — soluble fraction
Flash temperature, °C	265	266
Column temperature, °C	193	185
Detector temperature, °C	260	260
Helium pressure, lbs./sq. in.	12	12
Helium flow, ml/min.	60	60

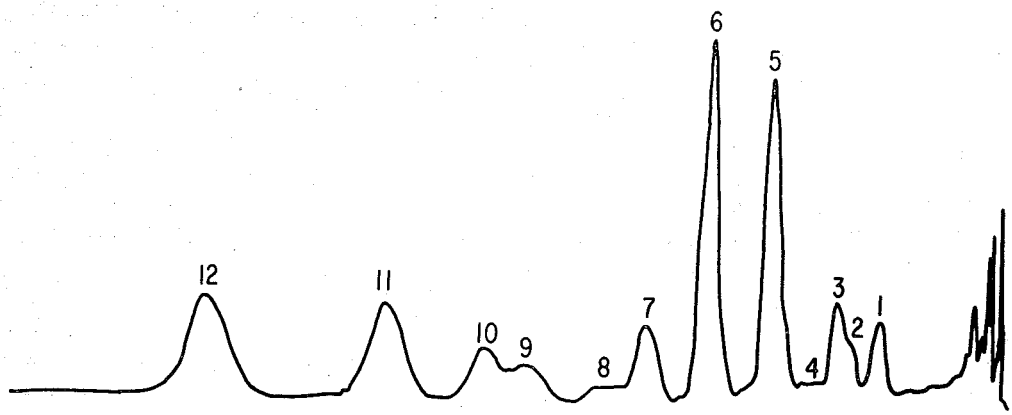

Fig. 1 — Analysis by gas-liquid chromatography of the methyl esters of fatty acids from the acetone-insoluble lipid fraction of the culture form of *Trypanosoma cruzi*. Length of analysis 25 minutes.

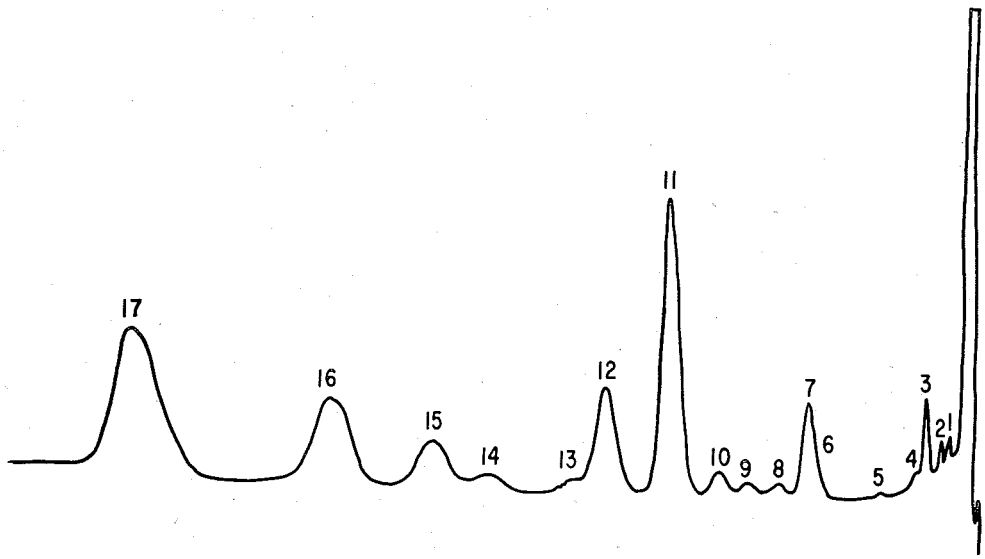

Fig. 2 — Analysis by gas-liquid chromatography of the methyl esters of fatty acids from the acetone-soluble lipid fraction of the culture form of *Trypanosoma cruzi*. Length of analysis 30 minutes.

TABLE 3

Analysis of methyl esters of lipids from *Trypanosoma cruzi* by gas-liquid chromatography

Carbon chain Length of Fatty acid	Number of double bonds	Acetone — insoluble lipids			Acetone — soluble lipids		
		Peak number (Fig. 1)	Weight percent	Relative retention time C 14 = 1.00	Peak number (Fig. 2)	Weight percent	Relative retention time C 14 = 1.00
C 8	—				1	0.14	0.19
C 9	—				2	0.14	0.24
C 10	—				3	1.4	0.33
C 10	1				4	0.11	0.38
C 12	—				5	0.05	0.58
C 13	—	1	3.3	0.75			
C 14 branched	—	2	2.0	0.94	6	0.54	0.93
C 14	—	3	4.0	1.00	7	4.3	1.00
C 14	1	4	0.8	1.17	8	0.7	1.15
C 15	—	5	16.7	1.40	9	1.0	1.33
C 15	1				10	1.5	1.49
C 16	—	6	22.7	1.76	11	19.8	1.80
C 16	1	7	5.7	2.11	12	10.1	2.14
C 17	—	8	1.4	2.37	13	1.4	2.36
C 16	2	9	5.2	2.82	14	10.3	2.85
C 18	—	10	6.6	3.09	15	6.6	3.14
C 18	1	11	13.6	3.56	16	13.2	3.72
C 18	2	12	18.2	4.69	17	28.7	4.78

It is evident that the above data are only a beginning. A goal for the future is the separation by preparative gas-liquid chromatography of sufficient amounts of the various acids as to allow confirmatory chemical characterization. Another very interesting point is the question whether the fatty acids are changed either quantitatively or qualitatively when the organisms are grown on different media. It should be noted in this connection that SHORB [20] found a parallelism between the fatty acids of the body and the medium in the case of *Trichomonas gallinae*.

NUCLEIC ACIDS

Desoxyribonucleic acid has been demonstrated in both the nucleus and the kinetoplast of *Trypanosoma cruzi* by PIZZI & DIAZ [19]. Subsequently, ribonucleic and desoxyribonucleic acid isolated from the culture form have been separated by FERNANDES & CASTELLANI [8] in whose papers (FERNANDES & CASTELLANI [8, 9]) numerous observations have been assembled on the acid-soluble adenine fraction, the nucleic acid adenine fraction, and the nucleic acid guanine fraction. Further evidence for the occurrence of both ribo- and desoxyribonucleic acid in the culture form of *Trypanosoma cruzi* has been presented by VON BRAND *et al.*[3] who identified in hydrolysates the nucleic acid bases adenine and thymine as well as ribose, and, with less certainty, cytosine and uracil. These investigators point out that part of the ribonucleic acid may be localized in the so called volutine granules which occur in profusion in the culture form. However, they have not yet been isolated and therefore any conclusion as to their chemical nature must for the present remain tentative. This has been emphasized strongly by ORMEROD [18] in respect to similar inclusion bodies observed in other species of trypanosomes.

AMINO ACIDS

The amino acids present in hydrolyzates of the culture form of *Trypanosoma cruzi* have been studied qualitatively and quantitatively by WILLIAMSON & DESOWITZ [28]. The following acids were identified and are expressed as moles percent: Cysteine + cystine/2, 0.1 ± 0.1; histidine, 4.1 ± 1.4; lysine + arginine, 14.4 ± 0.5; aspartic acid, 3.1 ± 1.0; glutamic acid, 20.8 ± 6.9; glycine, 9.1 ± 1.4; serine, 8.0 ± 0.3; alanine, 19.1 ± 1.2; threonine, 3.2 ± 0.4; valine, 8.7 ± 1.6; phenylalanine + leucines, 20.6 ± 0.8. In addition to the above acids, tyrosine and proline were demonstrated qualitatively, while the techniques employed did not allow the detection of tryptophane and methionine.

The same authors investigated also the free intracellular amino acids. They amounted to 5.2 ± 0.5 percent of the proteins present in the flagellates and alanine comprised 40 ± 1.5 percent of the free amino acids.

CONCLUDING REMARKS

The preceding pages show clearly how fragmentary our knowledge of the chemical composition of *Trypanosoma cruzi* is. On the other hand, it has been shown that at least some progress has been made in respect to the elucidation of the chemical constitution of several major fractions, indicating that sufficient material can be obtained to arrive at definite results through application of modern biochemicals methods. It is hoped that this account will contribute towards further development of this facet of the biochemistry of an important parasite.

RESUMO

Observações antigas e recentes sôbre a composição química do "Trypanosoma cruzi"

O autor reuniu os dados disponíveis sôbre a constituição química das formas culturais do *Trypanosoma cruzi*.

Quanto aos lípides, foram incluídos alguns resultados novos. A proporção de lípides é relativamente elevada, atingindo 20% do pêso sêco. Metade dêles, aproximadamente, é insolúvel em acetona e consiste de colesterol. Há, também, pequena quantidade de uma substância com espectro de absorção ultravioleta característico de um 5,7 dieno.

Os ácidos graxos não tinham sido estudados, até agora. As frações acetona-solúvel e acetona-insolúvel, após um processo de purificação-metilação foram re-esterificadas pelo método do trifluoreto de boro-metanol e analisadas por cromatografia em gás-líquido. As duas frações contêm grande variedade de ácidos graxos, dos quais muitos são comuns a ambas; mas os lípides acetona-solúveis contêm pequenas quantidades de ácidos em C 8 a C 12 que não são vistos na fração acetona-insolúvel. Esta última, por sua vez, tem menor percentagem de ácidos não saturados e uma proporção muito maior de um ácido saturado em C 15 do que a fração acetona-solúvel.

Foram revistos também os dados sôbre os carboidratos, os ácidos nucléicos e os amino-ácidos de *T. cruzi*.

Resumo do Editor

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