and that he always regretted that he had been drawn away by public business from more congenial pursuits. This may be true, but it is hard to believe that he would have chosen to spend his life manipulating glass tubes and Leyden jars in preference to managing men. There is given a very accurate and interesting account of the state of the science of electricity before Franklin, and a complete survey of Franklin's own work in the subject.

Then follows the text of those parts of Franklin's writings which have been selected by the editor to exhibit Franklin's discoveries in electricity, and a few letters on other subjects which show his scientific methods. An appendix contains some account of the work of Franklin's collaborators, particularly that of his friend and follower, Ebenezer Kinnersley: also an amusing anonymous letter addressed to Franklin in 1777, "in which his pretensions to the title of NATURAL PHILOSOPHER are considered." The critic chiefly objects that Franklin does not employ the methods of Sir Isaac Newton.

The book is beautifully printed at the Harvard University Press, and reflects great credit on its editor and publisher.

W. F. MAGIE

PRINCETON, N. J.

SPECIAL ARTICLES

THE EXTRACTION OF BIOTIN FROM TISSUES

THE interest in biotin and the biotin content of tissues has been great in recent months, partly because of the possible relationship of this substance to malignancy.[1,2,3]

The first determinations of the biotin content of various tissues and materials were made simply by studying the content of the hot water extracts.[4] Subsequently the freeing of relatively large amounts of additional biotin by the autolysis, for example, of liver tissue was observed.[5,6]

In an earlier publication[7] and in a recent bulletin[8] from this laboratory the biotin content of tissue autolysates was investigated, supplemented in the latter case by a few separate determinations on acid hydrolyzed specimens. The treatment used in these latter determinations is now recognized to be inadequate.

The purpose of this report is to bring together and to extend some of the observations made in this laboratory with regard to the question of different natural combinations in which biotin appears to exist.

Six methods of extraction were first investigated on eight tissues. The methods of extraction were (1) cold water (15 minutes), (2) hot water (100° C. 15 minutes), (3) autolysis (24 hours at 37° C. under benzene), (4) acid hydrolysis (6N H_2SO_4, autoclaved 1 hour at 15 lbs. pressure), (5) enzymatic digestion (1 per cent. "clarase" plus 1 per cent. "caroid," figured on the basis of the moist tissue, for 24 hours at 37° under benzene), and (6) enzymatic digestion for 48 hours, otherwise identical to (5). The tissues extracted were egg yolk, dialyzed egg yolk, dialyzed egg white saturated with biotin (avidin-biotin complex), rat liver, rat muscle, rat brain, *Pseudomonas fluorescens* cells, and *Clostridium butylicum* cells. After extraction the solids were removed by filtration through a thin mat of kieselguhr and the biotin content of the extracts determined by the method of Snell, *et al.*[5] The results are shown in Table I.

TABLE I

BIOTIN YIELDS BY DIFFERENT TREATMENTS (γ/GM. DRY WT.)

	Cold water	Hot water	Autolysis	Acid hydrolysis	24 hr. enzyme	48 hr. enzyme
Clostridium butylicum	.00048	.00098	.0046	.52	.62	.69
Pseudomonas fluorescens	.041	.068	.130	2.70	.77	1.19
Egg yolk	.018	.081	.088*	.41	.32*	.33*
Egg yolk, dialyzed	.029	.41	.27*	.50	.36*	.48*
Egg white-biotin, dialyzed	.16	4.90	.21†	8.10	.17†	.17†
Rat liver	.028	.056	.53	2.30	2.00	3.10
Rat brain	.0033	.0077	.0058	.27	.14	.22
Rat muscle	.0050	.0098	.0036	.11	.061	.098

* Heat sterilization was used in these and other cases not indicated.

† No heat sterilization was used in these cases.

It will be noted that acid hydrolysis under the conditions employed freed the maximum amount of biotin except in two cases in which enzymatic treatment yielded up to 35 per cent. more. Longer digestion with acid increases the yield (see Table II). If we consider the largest amount freed as the total amount present, the proportion freed by autolysis varies from 0.7 per cent. in the case of *Clostridium butylicum* up to 18 per cent. for rat liver. It is interesting that the *clostridium,* which is unable to synthesize

[1] P. M. West and W. H. Woglom, SCIENCE, 93: 525, 1941.

[2] W. L. Laurence, SCIENCE, 94: 88, 1941.

[3] V. du Vigneaud, Symposium on Vitamins, Chicago, September, 1941.

[4] F. Kögl and W. van Hasselt, *Zeits. physiol. chem.*, 243: 189, 1936.

[5] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Amer. Chem. Soc.*, 62: 175, 1940.

[6] György and coworkers found "vitamin H" freed by autolysis of yeast but not of liver. *J. Biol. Chem.*, 131: 733 and 745, 1939.

[7] R. E. Eakin, W. A. McKinley and R. J. Williams, SCIENCE, 92: 224, 1940.

[8] The University of Texas Publication, No. 4137, 1941.

biotin, does not contain autolytic enzymes to free it from combination. The relatively large proportion of biotin extracted from the egg white-biotin and the dialyzed egg yolk by hot water is notable.

From separate experiments not included in the table, it appears that biotin is much less completely freed from exhaustively dialyzed egg yolk by enzymatic treatment, than from undialyzed egg yolk. On the other hand, exhaustive dialysis of egg yolk renders biotin available for yeast in that even a cold water extract contains it in considerable amounts in an effective though non-dialyzable form. Dialysis of liver tissue does not render the biotin available (cold water), but enzymatic treatment of the dialyzed material frees the active substance to a considerable degree.

From the data in Table I it would appear that acid hydrolysis was in general the most effective extraction procedure. To study this further a series of acid and alkaline hydrolyses for different lengths of time were carried out on beef liver, beef heart muscle and *Clostridium butylicum* cells. The results of these experiments are recorded in Table II.

TABLE II

BIOTIN YIELDS BY ACID AND ALKALINE HYDROLYSIS (γ/GM. DRY WT.)

	Clostridium butylicum	Beef liver	Beef heart muscle
6N H_2SO_4, 120° C, 1 hr.	.84	2.90	.43
" " " 2 hr.	1.10	3.25	.49
" " " 5 hr.	.98	3.45	.49
" " " 10 hr.	1.05	2.90	.46
6N HCl, 120° C, 1 hr.	1.10	3.45	.46
" " " 2 hr.	1.10	3.35	.49
" " " 5 hr.	1.10	3.25	.43
" " " 10 hr.	.91	2.90	.44
6N NaOH, 120° C, 1 hr.	.77	2.55	.34
" " " 2 hr.	.49	1.50	.20
" " " 5 hr.	.32	.46	.09
" " " 10 hr.	.24	.35	.00
18N H_2SO_4, 120° C, 2 hr.	.74	2.90	.31

It will be seen immediately that alkaline hydrolysis is unsuitable, since it brings about a gradual destruction of the biotin. Sulfuric acid (6N) frees the maximum amount of biotin only after from two to five hours at 120° C. Hydrochloric acid (6N) appears to be somewhat more effective, the maximum amount of biotin being obtained after one or two hours. Some destruction of the biotin takes place with both HCl and H_2SO_4 on prolonged heating, although even with 18 N H_2SO_4, autoclaving for two hours results in a destruction of only from 20 to 40 per cent.

It appears that for many materials the surest method for extracting biotin consists in drastic acid hydrolysis, and on the assumption that the extraction is complete, the biotin content of rat and beef liver is about 3.5 γ per gram of dried tissue. This is about 1,000 times that originally found by Kögl and Hasselt[4] and agrees substantially with the values of West and Woglom.[1]

Biotin appears to occur naturally in different combinations which are broken down with varying degrees of ease. A study of these will be necessary before the functioning of biotin can be clarified.

ROY C. THOMPSON
ROBERT E. EAKIN
ROGER J. WILLIAMS

THE UNIVERSITY OF TEXAS

FLIES AS CARRIERS OF POLIOMYELITIS VIRUS IN URBAN EPIDEMICS[1]

THE recent accumulation of data suggesting that human poliomyelitis is primarily an infection of the alimentary tract with secondary localization in the central nervous system[2] has led to a renewed consideration of the possible role of flies in the transmission of this disease. Several groups of investigators have undertaken to search for the virus in flies caught during the course of various outbreaks of poliomyelitis this summer. Paul, Trask and their collaborators[3] have just reported the isolation of poliomyelitis virus from flies caught in 2 rural areas: (1) in a camp in Connecticut where several cases had occurred and (2) in Alabama near a privy used by three households where cases of poliomyelitis had recently occurred.[4] During the latter part of July and August we caught flies in 16 different urban sites during outbreaks of poliomyelitis in Atlanta and Cleveland. Because monkeys were not available the tests were not carried out till 10 to 12 weeks later, during which time the flies were kept in the frozen state in an insulated box containing solid CO_2. Although all the specimens have not yet been tested, we have already obtained 3 positive results: two with specimens caught in Cleveland and one with a small number of flies caught in Atlanta.[5]

The first specimen of flies to yield the virus has a rather interesting history. The site where the trap was set out was a government housing project consisting of modern, clean, thoroughly screened and hygienic homes situated on a hill in the center of Cleveland. There was a special brick enclosure for

[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

[2] A. B. Sabin and R. Ward, *Jour. Exp. Med.*, 73: 771, 1941; A. B. Sabin, *Jour. Am. Med. Asn.*, 117: 267, 1941.

[3] J. R. Paul, J. D. Trask, M. B. Bishop, J. L. Melnick and A. E. Casey, SCIENCE, 94: 395, 1941.

[4] Dr. John A. Toomey has just informed us that he has detected poliomyelitis virus in 2 specimens of flies caught in rural areas near Cleveland; one was trapped near an open privy 15 miles from the city and the other near a creek containing sewage just outside of town.

[5] Since this paper has been submitted for publication we have demonstrated the presence of poliomyelitis virus in two additional specimens of flies caught in two different regions of Cleveland during August 9 to 12 and August 14 to 16 respectively.