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.

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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.

the garbage cans, all of which were covered. Two children who developed poliomyelitis on August 7 and 9, respectively, were admitted to the City Hospital on August 11 from one of these homes. Investigation on August 16 revealed that 2 of 4 siblings had been ill for one day (August 4) with signs and symptoms suggestive of abortive poliomyelitis and that between August 7 and 13, 7 other children in the homes facing on the same yard had minor illnesses compatible with a diagnosis of abortive poliomyelitis. There was also the story that about a month before (early in July when only a few cases of poliomyelitis had been reported in Cleveland) after a severe storm the sewage overflowed, ran down the street, and some of the children became contaminated in the course of play. There were so few flies about that it hardly seemed worth while to set out a trap. However, about 500 flies (not identified as to species—mostly large green ones and many house flies) caught between August 16 and 18 yielded the virus upon inoculation into a Cynomolgus monkey. An etherized extract was injected intraperitoneally, and the unetherized material was given both intranasally and by mouth. The monkey developed paralysis on the 9th day and was sacrificed on the 10th day when all extremities were affected. Typical neuronal and infiltrative lesions were present in the spinal cord and passage to another Cynomolgus monkey resulted in flaccid paralysis on the 5th day and prostration on the 6th day. Histologically, this passage monkey exhibited necrosis and neuronophagia of practically all nerve cells in the spinal cord with typical infiltrative lesions. This virus was not pathogenic for mice, guinea pigs or rabbits.

The second positive result with a Cleveland specimen was with flies caught in the back yard of a private home (many miles from first site at the other end of town) in which 3 children of one family developed poliomyelitis between July 25 and July 30; two were removed to the hospital on July 30 and one on August 4. The home was fairly clean inside, with suitable toilet facilities, but it housed 7 other siblings, 2 of whom had had questionable minor illness. Four other children in adjacent homes gave histories of having had minor illness compatible with abortive poliomyelitis between August 1 and 10. There were open garbage cans in the neighbors' back yard and many flies were present. A large number of flies were caught in our trap, which was set out in the yard between August 9 and 12. Material from a specimen weighing 31 gm and consisting mostly of large green flies, some small house flies and one bee produced paralysis (confirmed by positive histological findings) in a Cynomolgus monkey on the 9th day.

The Atlanta specimen weighed only 2.5 gm and consisted of 203 small house flies, 3 green flies, 2 large black flies, one moth, 1 caterpillar and one unidentified 4-winged insect. It was collected between July 30 and 31 and represented a pool of insects caught in two places, one more or less in the center of town and the other on the outskirts. One case of paralytic poliomyelitis occurred in each home, but the children had been in the hospital since July 18 and July 14, respectively. The inoculated Cynomolgus monkey became paralyzed on the 15th day and exhibited poliomyelitic lesions in the spinal cord and medulla. Passage into another Cynomolgus monkey resulted in paralysis on the 6th day. The virus was not pathogenic for mice.

The ease with which poliomyelitis virus can thus be isolated from flies caught in urban areas (where immediate contamination with feces in open privies is at least not obvious) suggests that they may play an important role in transmission of the virus and may perhaps be responsible for the special seasonal incidence of the disease. Among the many problems which these findings raise for future investigation, the question of whether or not the virus may actually multiply in the flies deserves the most careful attention.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF MERCURY IN CONTACT WITH AMMONIA

IT has recently been called to the attention of the author that the use of mercury in contact with liquid ammonia or ammonia gas constitutes an explosive hazard, and that procedures involving the contact of these substances should be avoided. The author has been using mercury in contact with liquid ammonia and ammonia gas in various ways for more than twelve years. He has found mercury to be a most convenient substance for certain uses with liquid ammonia, and he has never found any combination of the two substances to be explosive under the conditions of his experiments. Several other workers known to the author have also used mercury and ammonia in contact without explosive reactions occur-