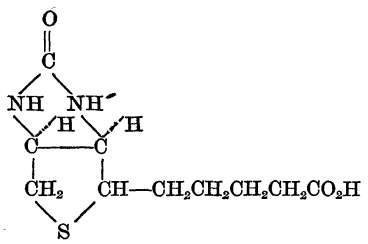
This structure possesses three different asymmetric carbon atoms which normally indicates eight optical isomers or four racemic modifications. However, it is believed from examination of models and reference to the literature[8,9] that two five-membered saturated heterocyclic nuclei fused in this manner can exist only in *cis* forms, as in Structure II.



II

trans Forms of the fused nuclei appear to involve strain which precludes their existence. Apparently, the synthesis of only one compound in which two five-membered rings fused in a *trans* manner through two adjacent atoms has been achieved, namely, *trans*-β-bicyclooctanone,[8] and this is carbocyclic. On this basis, only four isomers existing in two racemic modifications are indicated, and presumably biotin, which is optically active and optically stable,[10] is one of the four.

Any method of synthesis, therefore, has to take these factors into consideration. This we have done. Although the details of procuring best yields of desired intermediates, methods of resolution and other stereochemical problems, etc., are not completely worked out to our satisfaction for a detailed publication as a journal article, we wish to record at this time a comparison of our synthetic product with natural biotin. Synthetic biotin melts at 230–231°, which agrees with the recorded melting point,[11] and is identical with that of a specimen of natural biotin isolated by Dr. J. C. Keresztesy and kindly supplied by him. There is no depression of the melting point of a mixture. The rotation of the synthetic product, $(\alpha)^{25}_{D} + 90.7°$ (C = 2.0, N/10 sodium hydroxide solution) is in agreement also with that of the natural product, $(\alpha)^{25}_{D} + 91.4°$ determined here, and, $(\alpha)^{22}_{D} + 92°$, published[10] previously. The synthetic biotin crystallizes from water in long colorless needles and shows the same general solubility behavior as natural biotin. Its analysis follows: Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.12; H, 6.47; N, 11.23.

The authors wish to acknowledge the cooperation of Messrs. Glen E. Arth, Robert C. Anderson, Nelson R. Easton and Andrew N. Wilson, and Dr. Dorothea Heyl on the synthesis.

Results of assays on synthetic biotin, determined by Dr. J. L. Stokes, of the microbiology group, with *Lactobacillus arabinosus* No. 17–5 using a described medium,[12] modified to contain nicotinic acid but no biotin, shows that it has a biological activity equal to that of the natural biotin used as a standard.

Bioassays conducted by Dr. G. A. Emerson and Dr. W. H. Ott in the Merck Institute for Therapeutic Research with rats and chicks in which biotin deficiency had been induced by the feeding of egg-white-containing diets demonstrates the fact that the synthetic biotin produces a physiological response identical to that of natural biotin.

The results of the comparison of synthetic with natural biotin establishes their identity and, of course, proves unequivocally the assigned structure[6,7] of biotin.

STANTON A. HARRIS
DONALD E. WOLF
RALPH MOZINGO
KARL FOLKERS

RESEARCH LABORATORY,
MERCK & CO., INC.,
RAHWAY, N. J.

8 Linstead and Meade, *Jour. Chem. Soc.*, 935, 1934; Cook and Linstead, *ibid.*, 946, 1934; *ibid.*, 956, 1934.

9 Grigsby, Hind, Chanley and Westheimer, *Jour. Am. Chem. Soc.*, 64: 2606, 1942.

10 Melville, Hofmann and du Vigneaud, SCIENCE, 94: 308, 1941.

11 du Vigneaud, Hofmann, Melville and Rachele, *Jour. Biol. Chem.*, 140: 763, 1941.

12 Snell and Wright, *ibid.*, 139: 675, 1941.

PERSISTENCE OF YELLOW FEVER VIRUS IN THE BRAINS OF MONKEYS IMMUNIZED BY CEREBRAL INOCULATION[1]

PERSISTENCE of virus in the body of the host after infection, despite a refractory state to reinfection from without, has been shown to occur in the case of a number of the viruses, and it has been suggested that lasting specific immunity following some virus diseases depends on this persistence. Psittacosis[2] and salivary gland virus infection of guinea pigs[3] are classic examples of diseases in which such conditions have been encountered. Recovery of virus from the

1 From the Laboratory of the Yellow Fever Research Service, Rio de Janeiro, Brazil. The studies reported in this paper were carried out in the Rio de Janeiro laboratory of the *Serviço de Estudos e Pesquisas sobre a Febre Amarela* (Yellow Fever Research Service) which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

2 K. F. Meyer and B. Eddie, *Proc. Soc. Exp. Biol. and Med.*, 30: 483, 1933.

3 R. Cole and A. G. Kuttner, *Jour. Exp. Med.*, 44: 855, 1926.

brains of animals long after they had been inoculated and found to be refractory to reinoculation has been reported in encephalomyelitis of mice by Theiler,[4] and by Perdrau[5] in rabbits immunized with herpes virus.

Yellow fever vaccine is prepared in this laboratory with active attenuated virus, "17D" strain.[6,7] As a routine control procedure a sample of each lot of vaccine is inoculated into a rhesus monkey by the intracerebral route. Animals so inoculated occasionally show symptoms of central nervous system involvement such as paralysis and muscular incoordinations; even fatal encephalitis has been recorded, though rarely. Usually, however, as in the case of the animals comprising the present study group, the reaction observed is limited to a fever of short duration followed by recovery. Mouse protection tests performed with their blood serum collected thirty days after inoculation show specific neutralizing antibodies.[8]

Attempts were made to recover virus from the brains of some of these monkeys two to five months after inoculation. Three such animals died 63, 93 and 159 days after inoculation, apparently because of generalized tuberculosis. Intracerebral inoculation of mice with suspensions of brain material from these monkeys revealed the presence of an infectious agent capable of producing encephalitis in mice. Strains isolated from all three monkeys were identified immunologically as yellow fever virus. Although the virus in the original brain material was not titrated, a correlation between the period of its persistence and its concentration in the brain is suggested by the study of the mortality and the period of incubation of the inoculated mice. All of them were dead by the ninth and by the thirteenth days, respectively, following inoculation with material from the monkeys which died after sixty-three and ninety-three days. Brain material from the animal dying after 159 days, however, contained only sufficient active virus to produce encephalitis in three of the twelve mice inoculated. These were sacrificed for sub-inoculations shortly after becoming sick on from the eleventh to the thirteenth day.

No virus was recovered from the brains of five additional monkeys which were sacrificed approximately 100 days after inoculation.

An attempt was made in two monkeys, which had been inoculated with 17D virus 161 and 170 days previously, to localize the possibly persisting virus by injecting starch solution intracerebrally 10 days before killing the animals. No virus was isolated from either animal.

The possibility that the tubercle bacillus may play some role in unmasking a latent virus is suggested by the fact, already mentioned, that all three monkeys from which virus was recovered had died in the last stages of generalized tuberculosis.

H. A. PENNA
A. BITTENCOURT

SCIENTIFIC APPARATUS AND LABORATORY METHODS

NEW METHOD OF DETERMINATION OF THE CHOLINE-ESTERASE ACTIVITY

CHEMICAL methods to determine the activity of choline-esterase are based on measuring the quantity of acetic acid split off from acetylcholine by the enzyme. The manometric determination[1] of carbon dioxide is commonly used. In an alternate procedure the liberated acetic acid is titrated[2] directly with N/100 NaOH. The acetic acid has been determined also by the nephelometric as well as by the electrometric technique. We considered it worth while to compare the results obtained by the manometric and titration methods. The titration was modified as follows: 1–2 cc of human serum were diluted in a wide test-tube with distilled water to give 9 cc. Three drops of phenolphthalein were added and then N/100 NaOH until the solution turned to light red. Thereupon 1 cc of an acetylcholine solution (1:20) was introduced and the tube placed in a thermostat (38° C) for 20 minutes. The liberated acetic acid was then quickly titrated with N/100 NaOH. In comparing the above methods, human serum was used as the carrier of the choline-esterase, and the determinations were carried out simultaneously.

The results were in qualitative agreement when either undialized or dialized blood-serum was used. The same was the case when Prostigmin "Roche" was injected or taken orally before the blood-serum was obtained. Contrary to this, the results differed remarkably when a calcium chloride solution was added to the blood-serum. In the manometric method the calcium chloride appeared to exert a strong inhibitory effect on the choline-esterase, whereas in the titration method no influence of the calcium chloride was observed. Since the Ringer solution used in the manometric procedure contains calcium chloride, the following checks were carried out:

[4] M. Theiler, *Jour. Exp. Med.*, 65: 705, 1937.
[5] J. R. Perdrau, *Jour. Path. and Bact.*, 47: 447, 1938.
[6] M. Theiler and H. H. Smith, *Jour. Exp. Med.*, 65: 767, 1937.
[7] H. H. Smith, H. A. Penna and A. Paoliello, *Am. Jour. Trop. Med.*, 18: 437, 1938.
[8] J. P. Fox, *Jour. Exp. Med.* In press.
[1] R. Ammon, *Arch. ges. Physiol.*, 233: 468, 1933.
[2] Stedman, Stedman and White, *Biochem Jour.*, 27: 1055, 1933, modified by Hall and Lucas, *Jour. of Pharmacol.*, 59: 34, 1937.