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.

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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.

	cmm CO_2 liberated in 1 hour
Serum + Ringer solution + Acetylcholine	250
Serum + $NaHCO_3$ + Acetylcholine	325
Serum + Ringer solution + Acetylcholine + $CaCl_2$ (1/500 molar)	146
Serum + $NaHCO_3$ + Acetylcholine + $CaCl_2$ (1/500 molar)	234

It appears that the addition of calcium chloride prevents some of the carbon dioxide from leaving the solution. To prove, that the smaller amount of CO_2 found in the presence of calcium chloride was not due to an inhibitory effect of calcium chloride upon the choline-esterase, but to a methodical error, the acetylcholine was replaced by acetic acid in some determinations:

	cmm CO_2 liberated in 15 minutes
Serum + $NaHCO_3$ + N/100 acetic acid	129
Serum + $NaHCO_3$ + N/100 acetic acid + 0,1 cc $CaCl_2$ molar	120
Serum + $NaHCO_3$ + N/100 acetic acid + 0,2 cc $CaCl_2$ molar	95
Serum + $NaHCO_3$ + N/100 acetic acid + 0,2 cc $CaCl_2$ 1/100 molar	127
Serum + $NaHCO_3$ + N/100 acetic acid + 0,2 cc $CaCl_2$ 1/1000 molar	125

It follows from the above experiments that the titration procedure gives more reliable results, but it has the disadvantage that it can only be used in clear and almost colorless solutions.

Since the manometric method requires a Warburg apparatus we attempted to develop a more rapid procedure for the determination of the activity of cholinesterase in colored solutions. The following was found to be satisfactory: The serum, a $NaHCO_3$ solution and the acetylcholine solution were placed in the outer chamber of a Conway-jar[3] and a N/10 $Ba(OH)_2$-solution in the inner part. The total $Ba(OH)_2$ solution, or 0,5 cc was taken out 40 minutes after the Conway-jar was covered with the glass lid, and the quantity of unreacted $Ba(OH)_2$ was titrated with N/100 acetic acid. The values were satisfactory and in agreement with the results obtained by the titrimetric method mentioned above.

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CHEMICAL STUDIES ON CRYSTALLINE BARIUM ACID HEPARINATE

WE have obtained analytical data for the crystalline barium acid salt of heparin (barium acid heparinate) in comparison with those obtained for the neutral and acid salts of mucoitinsulfuric acid and chondroitinsulfuric acid. The following molar ratio was found for barium acid heparinate. Anhydrohexosamine : anhydrohexuronic acid : SO_3 : Ba = 2.0 : 1.9 : 6.0 : 3.0. Further, N : S : Ba = 2 : 6 : 3. Thus all barium is attached to ester sulfate and the carboxyl group of the uronic acid component is free. Summation (89 per cent.) of the above data, in comparison with the high summation (96 per cent.) obtained for the neutral sodium salt of chondroitinsulfuric acid, does not exclude the possible presence of another constituent. *d*-Glucosamine was identified (as *d*-glucosamine hydrochloride) in the hydrolyzate of the crystalline barium acid heparinate. Sodium heparinate (purified through the crystalline barium acid salt) consumes one mole (per 1,200 equivalent weight) of periodic acid.

The amino group of the *d*-glucosamine component of barium acid heparinate is not acetylated and is not free. Barium acid heparinate loses its anticoagulant potency on repeated crystallization from warm, dilute acetic acid. This change is accompanied by the appearance of a free amino group in the molecule, no sulfate is lost and the material is still stained with toluidine blue. Thus neither sulfate content nor toluidine blue staining power are true criteria of heparin activity.

Crystalline barium acid heparinate is also biologically inactivated by prolonged drying at elevated temperatures and by treatment ("Roche heparin" used in this experiment) with weakly ammoniacal hydrogen peroxide, the latter reaction resulting in appreciable sulfate loss.

Full details will be communicated at a later date.

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