fixation test for the diagnosis of several human encephalitides compels attention to the use of avirulent antigens, since certain active ones may possibly induce disease through accident or carelessness. Avirulent antigens can be prepared by means of ultraviolet light irradiation.[2] These antigens have the same antigenicity, specificity and lack of anticomplementary effect as fresh, virulent antigens. Moreover, they can be lyophilized and kept for a long time, up to two years.[3] However, inactivation with ultraviolet light requires special standardized equipment, which may not be available at all times and in all places.

Tests were made on the application of heat for preparation of avirulent antigens. The earlier "koktoantigens"[4] made by boiling brains suspensions for 30 minutes were inadequate since it was found in this laboratory that by this means the antigenicity was destroyed. By heating at 60° C for 30 minutes, however, avirulent antigens were obtained which retained sufficient antigenicity for practical use.

Ten to 15 cc of virulent antigens prepared as described,[3, 5] were placed in test-tubes and heated at 60° C for 30 minutes. During heating a more or less heavy flocculation took place. The flocculate was removed by centrifugation at 2,500 rpm for 10 minutes in a horizontal centrifuge, or at 6,000 rpm for 30 minutes in an angle head centrifuge. The supernate constituted the antigen, which could be lyophilized.

Heated antigens derived from the following viruses have been tested: St. Louis, Japanese B and Russian spring-summer (Far Eastern) encephalitis viruses; louping-ill and West Nile viruses; Western, Eastern and Venezuelan equine encephalomyelitis virus. The results of the tests are shown in Table 1.

The specificity of the heated antigens tested by cross-reaction was found to be unchanged and the same as that of unheated, virulent antigens. Furthermore, they had not acquired thereby any anticomplementary effect. The tabulated results show that although a loss of antigenic titer was correlated with heating, the antigenic titer after heating was sufficiently high for the viruses of Japanese B, Russian spring-summer and West Nile encephalitis for safe use. The heated antigens of louping-ill and Western equine encephalomyelitis viruses were somewhat lower but still usable; St. Louis and Eastern equine encephalomyelitis viruses could be employed sometimes, *i.e.*, if the initial titer could be made sufficiently high. The Venezuelan equine virus antigen after heating was ineffective. By test, lyophilization of heated antigens did not reduce their titer; they could therefore be handled for preservation or transportation in the same way as the irradiated antigens.

TABLE 1

ANTIGENS HEATED AT 60° C FOR ½ HOUR AND CENTRIFUGED

Antigen of virus of	LD_{50} titer before heating	Mouse inoc. with undiluted heated antigen	Complement-fixing titer of antigen	
			Before heat	After heat
St. Louis encephalitis	10^{-4}	0/10*	1:4	1:2
			1:16	1:2
Japanese B (Nakayama strain) encephalitis	$10^{-1.5}$	0/5	1:64	1:32
			1:128	1:16
	$10^{-3.5}$	0/10	1:16	1:8
			1:64	1:8
Russian spring-summer encephalitis	10^{-7}	0/5	1:16	1:8
	10^{-5}	0/10	1:32	1:16
Louping-ill	10^{-2}	0/10	1:16	1:4
West Nile disease	$10^{-4.5}$	0/10	1:64	1:32
Western equine encephalomyelitis	10^{-5}	0/5	1:64	1:8
	$10^{-6.5}$	0/10	1:32	1:4
Eastern equine encephalomyelitis	$10^{-6.5}$	0/10	1:2	0
			1:16	1:4
Venezuelan equine encephalomyelitis	$10^{-7.5}$	0/10	1:2	0
			1:8	0

* Fractions represent number of mice dead of virus infection over number used.

Summary. A simple method is described for producing avirulent mouse brain antigens for complement-fixation tests in human encephalitides, by application of heat at 60° C for 30 minutes. The heated antigens retain for most of the viruses employed a sufficiently high titer to warrant their use, since they are also specific, not anticomplementary, can be lyophilized and moreover, are avirulent, and thus can be handled with safety.

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THE RATE OF WATER LOSS FROM THE RESPIRATORY TRACT OF MAN LIVING IN A SUBTROPICAL CLIMATE[1, 2]

NEAR the turn of the century direct measurements of water loss from the lungs were made by Galeotti and his associates,[3] Weyrich,[4] Loewy and Gerhartz,[5]

and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army.

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[4] S. Nakagawa, *Z. Immun.-Forsch.*, 39: 563, 1924; J. Takaki, A. Bonis and O. Koref, *Z. Immun.-Forsch.*, 47: 431, 1926.

[5] J. Casals and R. Palacios, *Jour. Exp. Med.*, 74: 409, 1941.

[1] From the Department of Medicine, Tulane University, School of Medicine and Charity Hospital, New Orleans.

[2] Aided by a grant by the Rockefeller Foundation and Helis Institute for Medical Research.

[3] G. Galeotti and E. Signorelli, *Biochem. Zeitschr.*, 41: 269, 1912; *Arch. f. d. ges. Physiol.*, 160: 27, 1914–15; *Biochem. Zeitschr.*, 46: 173, 1912.

Benedict and Benedict[6] and more recently, in a limited number of subjects, by Seeley.[7] Most of these methods are cumbersome and difficult to use, and in no instance was the accuracy of the method adequately determined. Furthermore, the discrepancies in results rendered the data difficult to interpret.

A new and simple gravimetric method, condensing the expired water in cold aluminum coils for weighing, has been developed for the measurement of the rate of water loss from the respiratory tract of man.[8] The method has a mean accuracy of 0.3 per cent., range ±1.27 per cent., or a mean error of about 27 mg when collecting 1,000 mg (approximately the amount collected from each subject) of water.

In a study of 107 normal young adults of both sexes and White and Negro races (ages 17–43, all except four below 30 years) resting in a comfortable environment (room temperature 20.0–21.1° C.; relative humidity 55–60 per cent.) the rate of water loss from the respiratory tract was:

Mean, 0.878 ± 0.030 gram/m²/10 min.[9]
Range, 0.527 to 1.172 grams/m²/10 min.
Standard deviation, 0.333 ± 0.021 gram/m²/10 min.
Coefficient of variations, 37.90 ± 2.49 per cent.

There was no significant difference in sex or race.

The rate of water loss correlated highly with the rate of irrigation of the respiratory tract with air, the correlation coefficient being + 0.91 ± 0.02. A repetition of the studies in a hot month of August, 1944, in New Orleans and cool month of January, 1945, showed no significant seasonal differences. The rate and depth of respiration influenced the rate of water loss more or less in proportion to the rate of irrigation of the respiratory tract with air, although after correcting for the rate of irrigation of the lungs with air, the rate of water loss was greater when the respirations were slow and deep. Slow and deep respiration allows more time for evaporation of water into the inspired air. Exercise increased the rate of water loss, an influence due in a large part to the increased rate of ventilation of the lungs.

Cool foggy (temperature 15° C.; relative humidity 97 per cent.) or cool, dry (temperature 15° C.; relative humidity 60 per cent.) room air (inspired air) influence the rate of water loss relatively little while hot dry (50° C. and 18 per cent. relative humidity) increased the rate of water loss and a hot moist (50° and 49 per cent. relative humidity) reduced the rate of water loss considerably. The marked reduction in the water loss when inspiring hot moist air is due in a large part to the influence of cooling of air inspired (from 50° C. to about 39° C.) by the respiratory tract. This cooling at high temperatures increases markedly the relative humidity of the inspired air, thus reducing its capacity to hold more water. The relative magnitudes of absolute humidity of the air before and after inspiration and expiration are of more importance in understanding water loss than the relative humidity of either inspired or expired air considered separately.

When in a comfortable environment (*vide supra*) the mean temperature of the expired air was 33.19 ± 0.21° C., extremes 31.6 and 34.2 and the mean relative humidity 88.15 ± 1.31 per cent., extremes 78 and 96. The expired air, therefore, is not saturated. Its temperature and relative humidity varied with the conditions of air inspired. Inspiration of cool dry or cool foggy (*vide supra*) air influenced these very little. Inspiration of hot dry and hot moist room air (*vide supra*) influenced the temperature and relative humidity of the expired air considerably. The temperature of the expired air was greater than body temperature but cooler than the inspired air. The relative humidity of the expired air was lower than that when cool air was inspired. The degree of change was influenced by the warmth and wetness of the air inspired. For example, inspiring air at 50° C. and a relative humidity of 18 per cent. resulted in expired air at about 39° C. and about 76 per cent. relative humidity. When inspiring air at 50° C. and 49 per cent. relative humidity the expired air had a temperature of about 39.4° C. and relative humidity of about 74 per cent.

Therefore, the rate of water loss from the respiratory tract and the temperature and relative humidity of the expired air depend upon the conditions of the air inspired and the nature of respiration. Expired air is not and can not be saturated with water.

These studies of water loss and heat loss from the respiratory tract will be published in detail elsewhere.[8,9]

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[7] L. E. Seeley, *Trans. Am. Soc. Heat. Vent. Eng.*, 46: 259, 1940.

[8] G. E. Burch, "A Study of Water and Heat Loss from the Respiratory Tract of Man. Methods: I. A Gravimetric Method for the Measurement of Water Loss. II. A Quantitative Method for the Study of Heat Loss." To be published.

[9] The rates of water loss are all in grams per square meter of surface area of the body per 10 minutes.

[10] *Idem*, "Water and Heat Loss from the Respiratory Tract in Normal Subjects in a Subtropical Climate." To be published.