TABLE 2

DECREASE IN POTENCY OF LACTOGENIC AND THYROTROPIC HORMONES IN GROWTH HORMONE AFTER CYSTEINE TREATMENT

Preparation of growth hormone		Lactogenic M.E.D.* squab crop intramuscular	Thyrotropic M.E.D. squab thyroid intramuscular
		mg	mg
DAP35$_2$	Untreated	20	< 1
	Cysteine treated (total protein).	> 45	> 45
DAP74$_1$	Untreated	10	0.75
	Cysteine treated (soluble protein)	> 52	> 52

* Minimal effective dose.

From these results the following calculations can be made: 100 units[3] of untreated growth hormone contained from 5 to 10 units of lactogenic and from 100 to 150 units of thyrotropic hormone, whereas 100 units of cysteine-treated growth hormone contained less than one unit of either of these hormones.

Lactogenic, thyrotropic and gonadotropic hormones were next subjected to the same treatment with cysteine. Crude thyrotropic and gonadotropic hormones were found to be inactivated under the same conditions. The reduction of lactogenic hormone was accompanied by a precipitation. In a concentration above 0.1 per cent. this precipitation was almost quantitative, and no activity could be recovered from the precipitate or supernatant if a 40:1 ratio of cysteine to lactogenic hormone was allowed to react for two days at room temperature. Below 0.02 per cent. lactogenic hormone concentration, no precipitate was formed and no inactivation occurred under these same conditions. We are inclined to believe that the –SH form of the lactogenic hormone is as active as the native hormone and that its apparent inactivation is due only to its extremely low solubility.[4]

It has long been known that many organs which depend upon the pituitary for normal functioning may influence the body weight of animals. It is believed by others[5,6,7] that stimulation of these organs is essential for growth promotion by pituitary extracts or that growth is dependent upon synergism or addition of these effects. That the pituitary growth hormone has now been freed from two more (and possibly others) of the specific stimulating hormones of the pituitary seems of the greatest importance in establishing the individuality of this hormone.[8] A more detailed analysis of the action of cysteine on pituitary hormones and of the biological and chemical properties of the growth hormone obtained by this method will be published in the near future.

DONALD L. MEAMBER
HEINZ L. FRAENKEL-CONRAT
MIRIAM E. SIMPSON
HERBERT M. EVANS
UNIVERSITY OF CALIFORNIA

not appear to be quite as complete as inactivation of the other hormones. It is, however, true that fifty times the M.E.D. found in the untreated solution gave only a doubtful response.

[3] Growth unit defined in reference 1.

[4] While this work was in progress, it was learned from a remark by Bates in the discussion in the Cold Spring Harbor Symposium for Quantitative Biology (1938, p. 271) that he too had observed the inactivation of lactogenic, thyrotropic and gonadotropic hormones by cysteine.

[5] O. Riddle, Sigma Xi Lectures for 1936–7, Ohio State University Symposium on Hormones, p. 450.

[6] O. Riddle, *Endocrinology*, 19: 1, 1935.

[7] O. Riddle and R. W. Bates, *Endocrinology*, 17: 689, 1934.

THE CAUSATIVE AGENT OF INFECTIOUS EQUINE ENCEPHALOMYELITIS IN VENEZUELA

THE writers have been engaged in the study of the causative agent of infectious equine encephalomyelitis since the year 1936, when it made its appearance in Venezuelan Goajira. In this work, particular attention has been devoted to the etiological angle, which is, of course, of the utmost importance in the control of this disease. Some of the results obtained up to this date are briefly stated below.

(1) From the brain of animals which died of this infection in rural areas, a filterable virus has been isolated.

(2) This virus was found to be highly pathogenic to guinea pigs, whatever the mode of inoculation (intracerebral, intraperitoneal, intramuscular, subcutaneous), and caused them to die in from 48 to 72 hours after inoculation.

(3) Rabbits also proved to be very susceptible; they succumbed in from 72 to 96 hours after intracerebral inoculation.

(4) The disease could not be artificially transmitted to cattle, and this is in accordance with observations in rural localities, where bovines are refractory to the infection.

(5) The virus, carried again into horses, induces an acute condition with a clinical and anatomopathologic picture identical with that observed in animals attacked under natural conditions in rural areas. From the brain of horses thus inoculated, the original virus has been isolated.

(6) The virus is easily grown in chick embryos, which die in about 18 hours following inoculation. By means of successive passages, such a concentration was achieved that dilutions of 1:5.000.000 proved lethal to guinea pigs.

(7) The production of protective vaccine has been based on chick-embryo culture of the virus. Laboratory animals immunized with it withstand successfully

[8] H. M. Evans, *Proc. Assn. Res. Nerv. and Mental Dis.*, 17: 175, 1938.

thousands of lethal doses, a fact which proves the efficacy of the vaccine. For three months the Laboratories of Veterinary Bacteriology and Parasitology have been supplying the Ministry of Agriculture and Animal Husbandry of Venezuela weekly with considerable quantities of this autochthonous vaccine in order to combat the disease in rural areas.

(8) To determine the immunobiological characteristics of encephalomyelitic virus isolated in this country, the following has been employed:

(a) U. S. eastern strain virus;
(b) U. S. western strain virus;
(c) vaccine made in this country from the virus under observation (autochthonous);
(d) bivalent vaccine made from U. S. eastern and western strain viruses;
(e) encephalomyelitic serum made from autochthonous virus;
(f) U. S. bivalent encephalomyelitic serum made from both eastern and western strain viruses;
(g) U. S. monovalent encephalomyelitis serum made from western strain virus;
(h) Argentine encephalomyelitic serum made from Argentine virus.

From these comparative researches, carried out both *in vivo* and *in vitro*, it is to be inferred: (1) That the Venezuelan encephalomyelitic virus is wholly different both from the American western virus and from the Argentine virus, with which it has no immunobiological connection. (2) That it also differs immunobiologically from the American eastern strain virus, with which, however, it has some connection on account of its high virulence, pathogenicity, etc. (3) That the immunizing power of the protective vaccine made from autochthonous virus surpasses by far that of the American bivalent vaccine, prepared from both eastern and western viruses. (4) That the Venezuelan encephalomyelitic serum neutralizes the corresponding specific virus, not only *in vivo*, but also *in vitro*.

(9) As a result of these studies, the causative agent of infectious equine encephalomyelitis has been isolated for the first time in Venezuela. It is assumed that the agent in question constitutes a *sui generis* strain, different from the encephalomyelitis viruses described up to now.

(10) Venezuela should be given full credit for being perhaps the first South American country where the production of encephalomyelitic vaccine from embryo-cultured virus has been undertaken.

(11) The writers are about to finish a complete report on these researches which will be published in due course.[1]

VLADIMIR KUBES
FRANCISCO A. RÍOS
LABORATORIES OF VETERINARY BACTERIOLOGY AND PARASITOLOGY, MINISTRY OF AGRICULTURE AND ANIMAL HUSBANDRY OF VENEZUELA, CARACAS

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR STUDYING LIVING MOSQUITO LARVAE AND OTHER SMALL AQUATIC INVERTEBRATES

A VERY real problem in attempting to study small, living, aquatic animals under the microscope is to keep them in the field of vision without continual change of focus. The usual practices of entangling the animal in cotton threads or of treating the preparation with various drugs are generally undesirable, since the question naturally arises as to whether one is still dealing with a "normal" animal. There are, likewise, numerous objections to imprisoning the animal beneath a coverslip, because of the possible crushing of the preparation and the equally serious matter of cutting off its oxygen supply.

After numerous attempts to remedy this situation, the following very simple solution was found: A piece of thin copper wire (about No. 22 B. and S. gauge), six inches long, was bent at one end into a small circle one quarter inch in diameter, the ring of wire thus formed being made secure by several turns of the end of the wire around the stem of the loop. The other end of the wire was twisted around a small, square block of lead, such as is commonly used by histologists. The lead served as support for the wire pedicel and ring, as shown in Fig. 1.

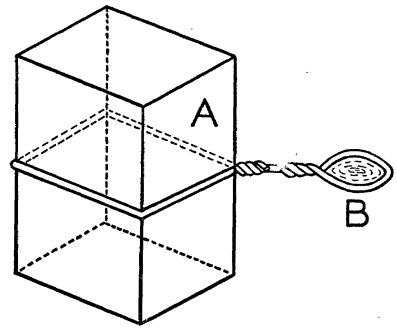

FIG. 1. Device for studying small, living, aquatic animals. *A*, lead block; *B*, wire loop with inclosed film of water.

[1] An already studied stock culture of our virus, with its virulence increased by consecutive passages through susceptible animals, has been supplied, for the sake of cooperation, to Lederle Laboratories, Pearl River, N. Y. C. E. Beck and Ralph W. G. Wyckoff, of these laboratories, published a communication on the subject in SCIENCE, 88: 2292, 530.