medium the cultures were found to be agglutinated in high titre by anti-A serum and to produce a neurotoxin which was completely neutralized by the "A" strain antitoxin. Further studies revealed that these microorganisms were inhabitants of the external surface of the eye, from which they could be cultured with great regularity, and frequently in large numbers unassociated with any bacterial colonies. It was apparent that as a result of the intraocular injection some of these microorganisms were carried into the eye, where they multiplied in the course of a few days. Similar studies carried out with mice of the Rockefeller Institute albino stock and of another Swiss strain (Freed) originating from the Institute stock but bred elsewhere for the past six to seven years, revealed the same condition, but the carrier rate was lower; it was thus possible to encounter groups of six mice from which these microorganisms could not be obtained, while in other groups of six, either all or a varying number yielded positive cultures of the same type. Evidence was also obtained that these microorganisms inhabit the mucosa of the nose and accessory sinuses from which they may be carried into the lungs in the course of nasal instillation under anesthesia.[4] It may be of interest to note here that in one test in which six mice were given nasal instillations under ether anesthesia and intraocular injections of pericardial fluid from a patient who succumbed to acute rheumatic carditis, three different types of pleuropneumonia-like microorganisms were isolated: a Type A from the eyes, a Type B from the lungs of one mouse, and a new type (to be called "C") which produced arthritis in mice but no toxin and was immunologically distinct from all the others studied. Two pleuropneumonia-like strains producing pneumonia in mice (isolated by Dr. Dienes in the course of passaging two human rheumatic heart muscle suspensions through the lungs of mice) were submitted to me for study[5]; they were found to be immunologically identical with Strain A and to produce neurotoxin in cultures which was neutralized by "A" antitoxin. Of three cultures isolated by Drs. Swift and Brown from pneumonic lungs of mice inoculated with rheumatic fever material, one was found to be a Type A, one a Type B and the third the same as the newly isolated Type C.

It is apparent, therefore, that the presence or absence of pleuropneumonia-like microorganisms in rheumatic fever and rheumatoid arthritis exudates and tissues will have to be established primarily by cultural methods, and not by passage through mice or other animals.

[4] This finding suggests that viruses, such as that of influenza, which are passaged by nasal instillation in mice, should be cultured periodically to determine whether or not they have been contaminated by microorganisms of the pleuropneumonia group.

[5] Dr. L. Dienes informs me that he was able to isolate a similar strain by passaging normal mouse lung.

Using a large variety of solid and fluid media and "passaging blindly" six or more times, I have been unable thus far to grow pleuropneumonia-like microorganisms from thirteen rheumatoid arthritic exudates (twelve patients), four rheumatoid subcutaneous nodules (three patients), rheumatoid synovial tissue (two patients), acute rheumatic blood, pleural fluid, pericardial fluid and heart muscle (two patients),[6] but many more cultivation experiments will have to be performed with suitable specimens before a final decision can be reached.

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THE PREPARATION OF PITUITARY GROWTH HORMONE FREE FROM LACTOGENIC AND THYROTROPIC HORMONES

THE knowledge that the anterior hypophyseal growth hormone is comparatively stable to alkali led us to assume that this protein would be free from disulfide linkages (–S–S–) and therefore resistant to cysteine reduction. Therefore a 5 per cent. solution of growth hormone in its purest form[1] was treated with double the amount of cysteine at pH 8.0. The reduction which resulted caused a precipitation of one half the total protein, whereas the supernatant contained most of the growth hormone when tested in normal plateaued female rats. (Table 1.)

TABLE 1

RETENTION OF GROWTH HORMONE POTENCY AFTER TREATMENT OF ANTERIOR HYPOPHYSEAL EXTRACTS $DAP35_2$ AND $DAP74_1$ WITH CYSTEINE

Preparation	Daily dose of total protein	Daily dose of soluble protein	Number of rats per group	Grams grain per rat*
	mg	mg		
$DAP35_2$ Untreated	1	1	9	39
$DAP35_2$ Cysteine treated	1	0.43	6	39
$DAP74_1$ Untreated	1	1	12	51
$DAP74_1$ Cysteine treated	1	1	5	43

* Average gain of normal plateaued female rats by 17 daily injections over a period of 20 days.

We tested the above-mentioned cysteine-treated growth hormone for its lactogenic and thyrotropic activity. Up to the very high levels tested, these hormones were found to be absent.[2] (Table 2.)

[6] I am deeply indebted to Dr. Edward F. Hartung, of the New York Post-Graduate Hospital, for supplying most of these specimens.

[1] Prepared by modification of the method published by Evans, Uyei, Bartz and Simpson (*Endocrinology*, 22: 483, 1938).

[2] As regards gonadotropic hormones: the follicle-stimulating hormone was known to be nearly completely absent from the untreated solutions, so tests for this substance were not considered necessary; inactivation of ICSH did

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.

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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.