the ratio of the activities toward these 2 substrates is similar to the enzyme in human serum. In addition, its activity is completely inhibited by eserine. Other studies(9) have shown that the substrate optimum characteristics are similar to the BuChE in serum, showing increasing activity with higher substrate concentration. All of these observations leave little room for doubt that the enzyme being measured in ruminant brain tissue is the characteristic BuChE found in nervous tissue from non-ruminant species. That this enzyme from ruminant tissue is incapable of hydrolyzing benzoylcholine in contradistinction to the enzyme from non-ruminant sources is of interest and this differential characteristic may lead to studies on the nature of the binding sites on the 2 enzymes which cause such differences in substrate specificity.

If this enzyme is found to be present in other species where it has hitherto been presumed to be absent, then its ubiquity in nervous tissue would imply that it may serve some basic function in metabolism in that organ.

Finally, it must be stated that the use of the terms acetylcholinesterase and butyrylcholinesterase is occasioned not by any conviction as to nomenclature, but rather that the terms hitherto employed have outlived their usefulness, and that some standardization in terminology is required. In this work, the specificity of each enzyme for chain-length optimum has been used as a basis for identification.

Summary. Studies on the cholinesterase in white matter of human, bovine, and sheep brain indicate that there is present in ruminant white matter a butyrylcholinesterase with properties similar to enzyme in human tissue, except for its inability to hydrolyze benzoylcholine. However, the enzyme from these 2 sources gives ratios of hydrolysis for butyrylcholine : acetylcholine which are alike, and the activity is completely inhibited by eserine. Thus it would seem justifiable to attribute to the enzyme a fundamental role in nervous tissue function, if its presence in other species can be confirmed.

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Influence of Cortisone and "Stress" upon Persistence and Multiplication of MEF_1 Virus in Hamsters.* (23325)

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It has been shown that under the influence of stress, the incidence of paralytic poliomyelitis in experimentally infected hamsters depends on endocrine reactivity to stress of individual animals(1). However, animals reacting to stress did not develop poliomyelitis when inoculated intraperitoneally unless proper inoculatory timing provided active virus during a specific stage of their endocrine stress-reaction(1). Observance of such timing requirements appeared to be particularly important for extraneural routes such as intraperitoneal, intramuscular, subcutaneous, etc., where the virus disappears within 3 days

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TABLE I. Persistence and Multiplication of MEF_1 Virus Introduced into Interscapular Brown Fat of Hamsters Treated with Cortisone or Submitted to Stress as Compared to Control.

Group	Viral titer of ISB* as tested in mice					
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr
Cortisone treated	3.4	4.5	5.4	5.9	†	†
Control animals	2.8	3.7	2.8	0	0	0
Sterile intraspinal inj.	2.8	3.8	3.4	1.4	0	0
Fixed on stretcher 6 hr	2	3.6	2.9	2.6	0	2.0
Refrigerated at 4°C 6 days	2.4	3.9	2.0	3.0	2.3	2.4

* Titers are expressed as negative log of the dilution producing a mortality of 50% in mice.
† Not determined.

following inoculation(1). In intracerebrally inoculated animals, where the virus persists 7-10 days, the chance of meeting the endocrine condition favorable for viral progression is considerably greater and, therefore, the importance of experimental timing is significantly reduced(1). Since the cerebral trauma involved in intracerebral inoculation was found to be a stress producing adrenal hypertrophy(1), it was thought that the long persistence of the virus following this route of inoculation may be partly a result of the accompanying stress. This study investigates the influence of various types of stress upon persistence and multiplication of MEF_1 poliomyelitis virus introduced into hamster interscapular brown fat, an extraneural tissue shown to be a site of poliomyelitis virus propagation(4). Normal and cortisone treated animals were used as parallel controls.

Materials and methods. Male Golden Hamsters 25-35 g were used. The interscapular brown fat (ISB) was surgically exposed and 0.1 ml of MEF_1 virus suspension of known infectivity titer was injected into it directly. At varying periods after inoculation the ISB was removed from groups of 3-6 animals, pooled, emulsified and its infectivity titrated in mice. Parallel series of stressed, cortisone-treated and normal hamsters were simultaneously prepared as above. Refrigeration, immobilization for 7 hours on a stretcher, and a pathogen-free intraspinal injection were used as stressing agents. Cortisone, 5 mg, was injected intramuscularly the same day as viral inoculation in the cortisone-treated groups. The viral titers shown in the Table represent the negative log of the dilution which when injected intracerebrally in 0.03 ml amounts in mice produced a 50% mortality. The LD_{50} dilution was calculated according to the method of Reed and Muench (2).

Results. Influence of cortisone: Brown fat of cortisone treated hamsters, removed at varying intervals after direct viral inoculation, showed significantly higher infectivity titers in mice than the brown fat of parallel controls (Table I). To compensate for possible presence of cortisone in the brown fat of cortisone-treated animals, the infected brown fat of controls was pooled with equal amounts of brown fat from non-infected but cortisone-treated hamsters, while the infected brown fat of the cortisone-treated group was pooled with non-infected brown fat taken from normal animals. The two resulting emulsions were diluted 1:500 in saline, then inoculated intracerebrally in 2 series of mice (Table II).

On the assumption that the pooling operation had eliminated possible differences in cortisone content, it was concluded that the difference in infectivity of the 2 emulsions demonstrated in Table II was due to a difference in viral concentration. Both Tables I and II indicate that, in addition to enhanced viral multiplication, viral persistence is significantly longer in cortisone-treated than in control animals.

Influence of stress: Table I shows that, when introduced into the brown fat of normal hamsters, MEF_1 virus progression follows a definite pattern of active multiplication during the first 48 hours followed by drop of infectivity titer and disappearance of the virus during the next 48 hours. In the brown fat of hamsters undergoing stress, viral multiplication follows the same pattern as in control animals for the initial 72 hours following inoculation. Beyond that time, however, in

TABLE II. Comparative Infectivity in Mice of Brown Fat Removed from Cortisone Treated and from Control Hamsters at Varying Intervals after Direct Inoculation with MEF_1 Virus.*

	Infectivity of brown fat as tested intracer. in mice								
	48 hr			96 hr			7 days		
Group	No. mice	% mortality	"P"	No. mice	% mortality	"P"	No. mice	% mortality	"P"
Emulsion "A"	50	94	0.0001	100	97	0†	100	12	0.01
Emulsion "B"	50	62		50	4		50	0	

* To compensate for possible presence of cortisone exclusively in the cortisone treated group the following tissues were pooled and emulsified: *Emulsion "A"*—50% infected brown fat from cortisone treated hamsters + 50% normal brown fat from non-infected hamsters; *Emulsion "B"*—50% infected brown fat from control hamsters + 50% non-infected brown fat from cortisone treated hamsters.

† Statistically highly significant.

contrast to the control where no virus could be detected, a significant viral titer was demonstrable in the brown fat of hamsters submitted to stress, indicating a longer viral persistence in the latter as compared to the former (Table I).

Discussion. From the above experiments it may be concluded that (a) cortisone or stress delayed significantly the disappearance of MEF_1 virus inoculated into the interscapular brown fat of hamsters, and that (b) in contrast with cortisone, which produced a prompt and significant enhancement of viral multiplication, stress did not induce any deviation from the multiplication pattern exhibited by controls during the initial 72 hours following viral inoculation. This difference in action upon viral multiplication seems to be responsible for the fact that although both cortisone and stress enhanced the total paralytic incidence, the former shortened(3) while the latter prolonged(1) the mean incubation period as compared to controls. Thus, shortening of the incubation period was closely associated with enhancement by cortisone of viral multiplication rate which accelerated the development of poliomyelitis in all susceptible animals. Stress, failing to enhance viral multiplication did not accelerate emergence of disease but, seemingly in consequence of its delaying action upon the disappearance of the inoculated virus, increased paralytic incidence by increasing frequency of belated paralysis (1). The increased number of belated paralytic cases increases the mean incubation time of the group as has been demonstrated previously(1).

Experiments in progress strongly suggest that the above difference between the action of cortisone and that of stress is a function of timing. Actually, in stress, although the end result is cortisone hypersecretion, the latter is preceded by pituitary ACTH discharge, which in recent, unpublished experiments, appears to exert as a side effect an action opposite to cortisone upon the metabolism of brown fat in hamsters. Endogenous ACTH has the same effect as the exogenous introduction, as suggested by metabolic alterations found in the brown fat of hamsters during pregnancy or during the early stage following stress. On the other hand, cortisone-like alterations(4,5) were found in the brown fat during the late stage following stress, which also supports the assumption that, in hamsters, cortisone is a relatively late-appearing product in the endocrine chain reaction initiated by stress. Possibly, a belated enhancement of viral multiplication subsequent to the belated hypercortisonemia initiated by stress is the mechanism by which disappearance of the virus is prevented in hamsters submitted to stress, in contrast with the controls.

Summary. 1) When MEF_1 poliomyelitis virus was introduced into the brown fat of hamsters treated with cortisone or submitted to stress there was viral persistence for periods of time significantly longer than in control animals. 2) During the initial 3 days following inoculation, viral multiplication rate was significantly enhanced in cortisone-treated but unchanged in stressed as compared to control hamsters. 3) The mechanism of the above difference as well as its effect upon the

development of experimental poliomyelitis is discussed.

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Effects of Protein Depletion and Inanition on Serum Glycoprotein Concentrations in the Rat.* (23326)

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Increases in the carbohydrate-containing proteins of serum have been reported in several pathologic states characterized by cachexia, such as cancer and tuberculosis(1). The suggestion that degradation of body tissues may be responsible for elevated serum glycoprotein levels(2) makes desirable experimental studies in which marked weight loss occurs but in which disease processes are not involved. If the catabolism of somatic tissues is responsible for increased serum glycoprotein levels, then nutritional states characterized by pronounced weight loss might be expected to cause an increase in the protein-bound carbohydrates of serum.

The present study is concerned with the effects of protein depletion and inanition on serum concentrations of total glycoprotein, seromucoid, albumin polysaccharide, globulin polysaccharide, total protein, albumin and globulin, and on the hemoglobin and hematocrit values of blood in adult, male, Sprague-Dawley rats.

Methods. The rats were housed singly in suspended type wire cages. They were maintained on a diet of Purina laboratory chow and tap water, *ad lib.* until initiation of the study. Blood samples were obtained by cardiac puncture under light ether anesthesia. *Normal Group.* The group was composed of animals maintained under the conditions described above. *Protein Depletion Group.* Rats were fed a protein-free diet† consisting of 70% corn starch, 15% alphacel (cellulose), 10% vegetable oil, 4% salt mixture (U.S.P. XIV), 1% cod liver oil, and supplemented with the following vitamin mixture† (mg/kg): alpha tocopherol 110, ascorbic acid 1000, inositol 110, choline chloride 1660, menadione 55, p-aminobenzoic acid 110, niacin 100, riboflavin 22, pyridoxine hydrochloride 22, thiamine hydrochloride 22, Ca pantothenate 67, biotin 0.44, folic acid 2, Vit. B_{12} 0.03, Vit. A 20,000 units/kg, and Vit. D 2,220 units/kg. The animals were fed the diet 20 days at which time they had lost approximately 25% of their original weight. *Inanition Group.* Food was withheld from the rats until they had lost 25% of their original weight, the depletion requiring 8 days. Drinking water was available at all times. *Serum and blood determinations.* Chemical and hematologic analyses were carried out by methods previously reported(3,4,5). The globulin fraction was isolated by an adaptation of the method of Pillemer and Hutchinson(5). The components of the albumin fraction were estimated by difference. An

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