TABLE I. Serum Lipoproteins, Determined at Density 1.21, Cholesterol, Total Lipids and Total Proteins of 13 Rats with the Nephrotic Syndrome.

Duration of disease	Severity of disease	Total protein, g/100 ml	Cholesterol, mg/100 ml	Total lipid, mg/100 ml	-S>70	40–70	25–40	10–25	1–10
					mg/100 ml				
Control	—	5.2	60	360	99	—	26	20	114
"	—	5.2	75	440	39	—	20	38	91
16 mo	2+	5.96	75	1600	33	—	21	—	129*
13	3+	5.83	105	1460	42	21	33	106	152
12	4+	4.88	140	1680	164	19	71	141	223
11	4+	5.29	208	2360	77	24	75	141	182
10	4+	5.17	242	1880	282	38	59	106	152
7	4+	5.63	270	1900	398	—	60	86	315*
7	4+	3.42	550	3310	—§	282	282	—	101*
4 wk	4+	4.63	155	4720	170	—	34	57	199
17 days	4+	2.96	485	5880	552‡	40	152	—	199*
17	4+	3.29	400	5240	>400	—	129†	71	168
17	4+	3.96	244	2650	282‡	16	47	164	164

* Spreading peak -S 1-15. † No clear resolution -S 25-70. ‡ -S 70-400; -S>400++++.
§ -S>400++++.

disease resulting from desoxycorticosterone acetate priming followed subsequently by renin injection(7). This may be due to differences in extensiveness of the lesions or duration of the disease.

Summary. Ultracentrifugal analyses at a density of 1.21 of serum lipoproteins in the nephrotic syndrome of rats induced by the intravenous injection of N.T.S. obtained from rabbits, showed greatly increased concentration of all lipoprotein components but chiefly in the lower density fractions. The amount of change was not directly correlated with the duration of the disease, but was generally comparable to its severity. The changes observed in the rat were similar in type to those in the nephrotic syndrome of children.

1. Heymann, W., and Lund, H. Z., *Pediatrics,* 1951, v7, 691.

2. Longworth, L. G., and MacInnes, D. A., *J. Exp. Med.,* 1940, v71, 77.

3. Lewis, L. A., and Page, I. H., *Circulation,* 1953, v7, 707.

4. Gofman, J. W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., and Herring, V., *Science,* 1950, v111, 166.

5. Lewis, L. A., Green, A. A., and Page, I. H., *Am. J. Physiol.,* 1952, v171, 391.

6. Gofman, J. W., Lindgren, F., Jones, H. B., Lyon, T. P., and Strisower, B., *J. Gerontol.,* 1951, v6, 105.

7. Lewis, L. A., Masson, G. M. C., and Page, I. H., *Fed. Proc.,* 1954, v13.

Received July 19, 1954. P.S.E.B.M., 1954, v86.

Participation of Brown Fat in Pathogenesis of Experimental Poliomyelitis of Monkeys.* (21228)

GREGORY SHWARTZMAN AND STANLEY M. ARONSON.

From the Department of Microbiology, Mount Sinai Hospital, New York City.

Progression studies have served to indicate some of the pathways by which peripherally administered poliomyelitis virus (Type 2) reaches the CNS in cortisone-treated hamsters (1-3). Somatic lesions in two types of tissues were noted during the anteneural phase of the experimental disease. A myositis was demonstrable(4) as well as lesions in brown (hibernating) fat(5). High concentrations of virus were present in these foci. The following studies deal with the possible role of brown

* Aided by a grant from the National Foundation for Infantile Paralysis.

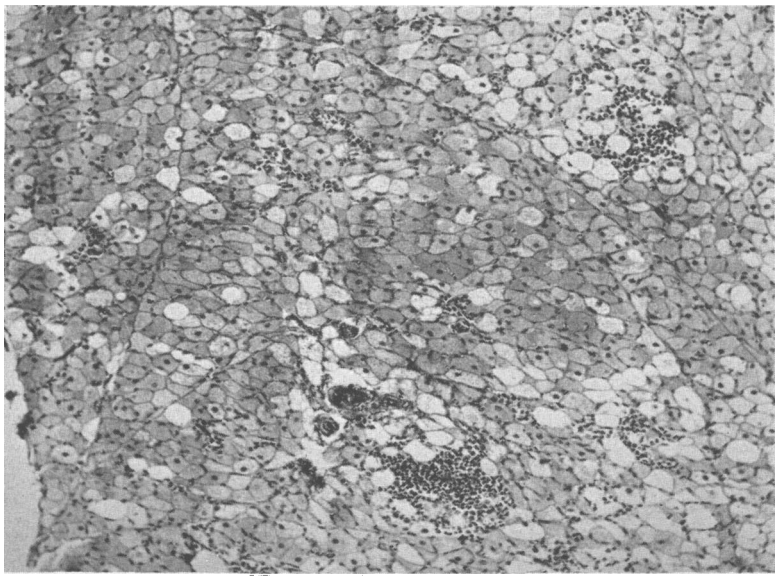

FIG. 1. Biopsy of brown fat resected from left axilla, 5 days after animal was inoculated intramuscularly into the left arm with Wisconsin strain (Type 1). Animal developed paralysis 3 days after biopsy. There are numerous islands of cellular necrosis and leucocytic infiltration. On subsequent intracerebral and peripheral passage the tissue produced typical poliomyelitis. H. & E. × 100.

fat in pathogenesis of experimental poliomyelitis in the monkey.

Experimental. Young male cynomolgus monkeys were used in all experiments. Three strains of Type 1 poliomyelitis virus (MacMahon, Wisconsin, and Brabyn, isolated from human stool and typed by Dr. Robert Ward) were available for study. The inoculum consisted of centrifuged monkey cord emulsion, diluted 1:50 and injected in a volume of 1 cc. Cortisone was administered intramuscularly into hind limbs in daily dosage of 10 mg for a period of 7 days. Following the fourth injection of cortisone each strain was inoculated intramuscularly into the left arm of 2 monkeys. After a period of 5 days, biopsies of the ipsolateral axillary brown fat were taken from each animal under barbiturate anesthesia. A portion of each resected specimen was studied histologically and the remainder used for animal passage. For the latter purpose, the tissue was ground with sterile sand and mixed with saline solution to give a final dilution 1:50. The suspension was centrifuged at 1500 rpm for 5 minutes and the supernatant filtered through sterile Whatman filter paper (No. 1). The filtrate contained no gross particles and appeared opalescent. Animals infected with Wisconsin and MacMahon strains were sacrificed on the first day of paralysis and those inoculated with the Brabyn strain were killed the day following biopsy and prior to clinical paralysis. Complete autopsies were performed on all monkeys. Biopsied brown fat suspensions were passaged serially in cortisone-treated and untreated monkeys by intracerebral and intramuscular routes. Selected material was inoculated into tissue cultures of monkey renal epithelium(6). Axillary tissues other than brown fat obtained at autopsy were also inoculated into monkeys. The anatomy and histology of brown fat was studied in normal, cortisone-treated and infected monkeys.

Nature and distribution of brown fat in the cynomolgus monkey. Brown fat differs from ordinary adipose tissue in many respects. The former is lobulated, demarcated, confined to specified anatomic regions and dissimilar in biochemical constitution(7,8). Brown fat cells are smaller and contain centrally located nuclei. The cytoplasm contains small polyloculated lipid vacuoles separated by strands of eosinophilic matter. The tissue is

TABLE I. Serial Passages of Infected Brown Fat in Monkeys.

Animal No.	Inoculum	Site of inoculation	Cortisone	Day of onset of paralysis	Autopsy findings
I	Wisconsin monkey cord	I.M., left arm	+*	8	Poliomyelitis
II			+	6	"
III	MacMahon monkey cord		+	7	"
IV			+	12	"
V	Brabyn monkey cord		+	—	S† on 6th day. CNS normal
VI			+	—	" " 7th " . " "
VII	Preparalytic axillary brown fat biopsy monkeys I, II		+	7	Poliomyelitis
VIII		I.C.	+	5	"
IX	Postparalytic axillary brown fat (autopsy) monkeys III, IV		+	7	"
X	Preparalytic axillary brown fat biopsy monkeys V, VI		+	6	"
XI	Spinal cord, monkey VIII		+	5	"
XII	Tissue culture suspension infected with preparalytic axillary brown fat biopsy tissue from monkeys I, II	I.M., left arm	+	5	"
XIII			+	6	"
XIV			—	6	"
XV		I.C.	+	3	"
XVI			—	6	"
XVII			—	3	"
XVIII	Postparalytic axillary brown fat (autopsy) monkey VII	I.M., left arm	+	7	"
XIX			—	4	"
XX		I.C.	+	6	"
XXI			—	6	"
XXII	Postparalytic axillary white fat (autopsy) monkey VII	I.M., left arm	+	—	S after 25 days. CNS normal
XXIII	Postparalytic axillary lymph node (autopsy) monkey VII		+	—	*Idem*
XXIV			+	—	"
XXV	Postparalytic axillary nerve trunk (autopsy) monkey VII		+	7	Poliomyelitis

* 10 mg/day for 7 days. † S = Sacrificed.

endowed with a rich capillary circulation. The principal depot of simian brown fat was observed in the axillae in the form of adjoining lobules. A small amount of this tissue was also found between the posterior and lateral cervical musculature, as well as in the scapular region. Minute amounts were detected in the thoracic paravertebral regions and in the soft tissues encompassing the adrenal glands.

Results. Effect of cortisone upon brown fat of the cynomolgus monkey. Two cynomolgus monkeys were inoculated daily with 10 mg of cortisone for a period of 7 days and sacrificed 1 day after the last injection. Two control monkeys of similar age were sacrificed at the same time. Masses of brown fat from each of the 4 animals were dissected out, fixed in buffered formalin solution and studied histologically. The polylocular configuration of brown fat cytoplasm was unusually prominent in cortisone-treated monkeys, and the cells visibly enlarged. Cell diameters were determined for 500 brown fat cells from each specimen. The mean diameter of brown fat cells of control animals was 16.4 $\pm$ 0.15 μ, while that of the cortisone-treated animals was 25.3 $\pm$ 0.64 μ. No inflammatory or degenerative features were visible in the microscopic sections of brown fat obtained from control and cortisone-treated monkeys.

Histological changes and viral proliferation in brown fat. Five to 7 days following viral inoculation into the upper limb muscles, multiple isolated lesions appeared in the ipso-

lateral axillary brown fat (Fig. 1). Lesions of approximately equal severity followed inoculation of all strains used. The primary changes consisted of fragmentation, beading and coarseness of the cytoplasmic strands. The degenerating nucleus was displaced peripherally. In early lesions small numbers of polymorphonuclear leukocytes were often present in the vicinity of necrotic cells. At a later phase of the process, inflammatory cells obscured the focus of necrosis. Perivascular inflammatory infiltration was seen in neighboring regions. In the terminal phase of the disease, lesions were also observed in the contralateral axillary and paravertebral deposits of brown fat.

As may be seen from Table I, axillary brown fat, resected during the preparalytic period from animals I and II infected with the Wisconsin strain, was inoculated into monkeys VII and VIII intramuscularly and intracerebrally. The animals developed paralysis on the 7th and 5th days, respectively. The cords from monkeys VII and VIII showed histologically typical poliomyelitis and the cord from monkey VIII induced the disease on intracerebral passage (monkey XI). Titration of the brown fat emulsion in tissue cultures revealed a cytopathogenic titer in the order of 5 logs. The tissue culture fluid was then inoculated into cortisone-treated and untreated monkeys, intramuscularly (XII-XIV) and intracerebrally (XV-XVII). All 6 monkeys developed paralysis within 6 days.

The contents of the axillary cavity of monkey VII were carefully removed and separated into component tissues. The resected brown fat was inoculated into cortisone-treated and untreated monkeys, intracerebrally (XX, XXI) and intramuscularly (XVIII, XIX). All 4 monkeys showed paralysis within 7 days. Axillary white fat was injected intramuscularly into cortisone-prepared monkey XXII. The animal failed to demonstrate any paralytic symptoms during the 25 day period of observation and showed no histological lesions. Axillary lymph nodes were inoculated intramuscularly into cortisone-treated monkeys XXIII and XXIV. No paralysis ensued and the CNS was histologically normal. Brachial plexus nerve trunks were passaged intramuscularly into cortisone-treated monkey XXV. This animal became paralyzed in 7 days and autopsy confirmed the presence of poliomyelitis.

Two monkeys (V, VI) inoculated intramuscularly with Brabyn strain were sacrificed 6 days following inoculation, prior to the development of paralysis. Lesions were confined to the ipsolateral axillary brown fat and the CNS showed no evidence of poliomyelitis. Brown fat was inoculated intracerebrally into a cortisone-treated monkey (X) which showed severe paralysis in 6 days. Autopsy showed typical poliomyelitis.

Two cortisone-treated monkeys (III and IV) infected intramuscularly with MacMahon strain were sacrificed the day of paralysis and the ipsolateral axillary brown fat was inoculated intracerebrally into monkey IX. This animal succumbed in 7 days and histologic sections of the brain stem and spinal cord showed extensive lesions of the anterior horns and motor segments of the medulla.

Discussion. In the cortisone-treated Syrian hamster, the preparalytic period is characterized by demonstrable somatic lesions, namely, myositis and necrosis of brown fat. The muscle lesions are morphologically indistinguishable from those induced by Coxsackie virus(9). Extraneural propagation of the poliomyelitis virus in muscles is probably unique to the hamster since we have been unable to demonstrate this phenomenon in mice or monkeys. There also occurs in the cortisone-treated hamster a marked proliferation of the virus in brown fat, commencing 2-3 days prior to neuraxial involvement and accompanied by severe necrobiosis. It is of interest to point out that most strains of Coxsackie virus show a similar lipotropic property in suckling mice, eventuating in lesions remarkably like those produced by Type 2 strains of poliomyelitis virus in the hamster(9).

While brown fat is more prominent in hibernating animals, it is nevertheless demonstrable in most mammalian species. In the monkey, the principal deposit is located in the axillary apices. Simian brown fat is essentially similar to rodent brown fat as determined by histologic and histochemical pro-

cedures. In addition, monkey brown fat undergoes hypertrophy under cortisone treatment in the same manner as does rodent brown fat(10).

Studies described in this paper indicate that following intramuscular inoculation into cortisone-treated monkeys, the virus is capable of proliferation in brown fat prior to CNS involvement, thus suggesting that under our experimental conditions the brown fat constitutes an extraneural site of viral multiplication. Detectable quantities of virus persist in this site during the paralytic stage, as well. Cortisone was used in subsequent passages to insure successful takes following parenteral introduction of the virus(11). It is pertinent to point out, however, that in subsequent passages the use of cortisone is not obligatory for production of steatitis, viral propagation in brown fat and CNS involvement. It remains to determine whether viral multiplication in brown fat on subsequent passages, in the absence of cortisone, was due to adaptation of the virus to this tissue.

It is of note that the widespread necrotic and inflammatory lesions in brown fat invariably coincide with viral propagation.

Summary. Following peripheral inoculation, poliomyelitis virus (Type I) proliferates in the brown fat of cortisone-treated monkeys during the preparalytic and paralytic stages of the disease. On subsequent peripheral passage of infected brown fat, the virus can be detected in the brown fat in the absence of cortisone treatment. Characteristic brown fat lesions are incidental to viral proliferation.

The authors are indebted to Dr. René Jahiel for the tissue culture studies and to Mr. Guido Rio for capable assistance.

1. Shwartzman, G., and Aronson, S. M., *Fed. Proc.*, 1953, v12, 458.

2. ———, PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 573.

3. ———, Bela Shick Lecture, *J. Mt. Sinai Hosp.*, 1954, v21, 3.

4. Aronson, S. M., and Shwartzman, G., *A.M.A. Arch. Path.*, 1953, v56, 557.

5. Aronson, S. M., Shwartzman, G., and Teodoru, C. V., *J. Neuropath., and Exp. Neurol.*, in press.

6. Melnick, J., *Am. J. Public Health*, 1954, v44, 571.

7. Fawcett, D. W., *J. Morph.*, 1952, v90, 363.

8. Menschik, Z., *Anat. Rec.*, 1953, v116, 439.

9. Pappenheimer, A. M., Daniels, J. B., Cheever, F. S., and Weller, T. H., *J. Exp. Med.*, 1950, v92, 169.

10. Aronson, S. M., Teodoru, C. V., Adler, M., and Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 214.

11. Shwartzman, G., and Aronson, S. M., *The Effect of ACTH and Cortisone upon Infection and Resistance,* Chap. 14, Columbia University Press, 1953.

Received July 20, 1954. P.S.E.B.M., 1954, v86.

In vitro Cultivation and Cytopathogenicity of Vesicular Exanthema Virus.* (21229)

MARY E. MCCLAIN,† STEWART H. MADIN, AND PAUL C. ANDRIESE.
(Introduced by R. S. Muckenfuss.)

From the Department of Bacteriology, University of California, Berkeley.

The epizootic of vesicular exanthema of swine which spread over most of the United States in 1952-1953 brought to general attention the lack of information concerning the etiological agent. Although isolated as early as 1934(1), this virus has received comparatively little attention from virologists due, in part, to the consistent failure to propagate the agent with ease in any species except swine. The present report describes the first successful cultivation of vesicular exanthema virus

* Grateful acknowledgement is made to Miss Jane Seely and Miss Doris Clinger for their valuable technical assistance. We are particularly indebted to Dr. J. H. Dirsten for his unfailing efforts to supply the swine embryo materials.

† Present address: Virus Laboratory, University of California.