TABLE I.
Liver Glycogen in Normal and Diabetic Rats (Fasted 24 Hr).

Status	No. of animals	Liver glycogen, mg/100 g liver	P	Blood sugar, mg% (mean and range)
Diabetic	11	.65 ± .14	<.01	658 (403-1100)
Normal	13	.08 ± .03		
Non-diabetic (received alloxan)	6	.14 ± .07	>.4	129 (120-143)

TABLE II.
Liver Glycogen Following Administration of Adrenal Cortical Extract to Diabetic and Non-diabetic Rats. (All animals adrenalectomized and fasted 32 hr).

Status	No. of animals	Treatment	Liver glycogen, mg/100 g liver	P	Blood sugar,† mg% (mean and range)
Diabetic	14	0	.012 ± .002	>.2	410 (264-620)
Non-diabetic	17	0	.016 ± .003		
Diabetic	7	ACE*	.44 ± .08	>.9	380 (280-645)
Non-diabetic	9	ACE*	.43 ± .10		
Diabetic	9	ACE	1.67 ± .16	>.2	397 (296-556)
Non-diabetic	10	ACE	1.97 ± .16		

* Diluted 1:1. † Prior to adrenalectomy.

glycogen response to adrenal cortical extract of diabetic rats was found not to be significantly different from the response of non-diabetic rats. Both groups were adrenalectomized and fasted 32 hours.

Received November 3, 1949. P.S.E.B.M., 1949, v72.

Hemagglutination and Complement Fixation with Type I and II Albany Strains of Coxsackie Virus.* (17524)

JORDI CASALS, PETER K. OLITSKY, AND LESLIE C. MURPHY†

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

Dalldorf and Sickles(1) isolated a virus from the feces of patients having symptoms resembling those of abortive or even paralytic poliomyelitis. This active agent, characterized by pathogenicity for suckling but not for adult mice, was later named Coxsackie virus.(2) Melnick, Shaw, and Curnen(3) recovered several similar viruses some of which they found to be distinct by the neutralization test. Dalldorf(4) also determined such specificity for his strains, here designated as Type I and Type II Albany strains. The difficulties of working with infant mice are

* With the technical assistance of Joan Fitz-Gerald and Barbara Quinn.

† Major, V.C., U. S. Army.

1. Dalldorf, G., and Sickles, G. M., *Science*, 1948, v108, 61.

2. Gifford, R., and Dalldorf, G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 589.

3. Melnick, J. L., Shaw, E. W., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 344.

4. Dalldorf, G., personal communication.

considerable; we were thus led to a study of a possible *in vitro* reaction that might be applicable for the identification of the virus, namely, hemagglutination or complement fixation. The present report concerns itself with the results of studies of the two reactions employing 2 of the Albany strains, Type I (T.T.) and Type II (Fleetwood), generously presented to us by Dr. Dalldorf.

Hemagglutination Tests. Agglutination tests were carried out in a manner already described,(5) except that the saline solution used for dilution of materials was buffered with 0.02M, instead of 0.05M phosphate. Human group O, sheep, chick, and guinea pig RBC were employed and the two different strains, Type I and II, of the Coxsackie virus. In addition, two forms of each strain were studied; suspensions of infected brain and of limbs, derived from infant mice which had reacted after their injection intraperitoneally of either active brain or limb virus.(6) The tubes containing materials were held at 5, 23, and 37°C and read at intervals up to 2 hours. As an indicator of the possibility of the materials yielding positive reactions, a test was set up with sheep cells and MM virus; definite agglutination was seen in 1:160 dilution of the virus after 1 hour at 5°C.(5) The results of all the hemagglutination tests with the 2 strains of Coxsackie virus were, however, negative. It should be stressed that suspensions of normal mouse brain, especially of fresh brain, if not properly centrifuged, show agglutination of sheep RBC, the degree of hemagglutination depending on the particles in the supernate.

Complement-fixation tests. Complement-fixation tests have been carried out with the same Albany strains using mouse immune serum and antigens derived from infected brain or limb tissue of suckling mice, Swiss-W strain.‡

Immune Serum. Antisera were prepared in 3- to 4-month-old Swiss-W mice by giving each of them, intraperitoneally, 0.5 ml of mouse-brain virus, 10^{-1} dilution, on the 1st and 5th day; they were bled on the 15th day. These animals were then injected intraperitoneally with limb virus, each with 0.5 ml of 10^{-1} dilution, on the 19th and 25th day; they were bled again on the 35th day. In all 20 mice were so treated with each type of virus.

Antigen for test. Antigens for complement-fixation tests were prepared following a method previously described (acetone-ether extracted antigens).(7) In the present study, 2- to 11-day-old Swiss mice were given .05 ml of 10^{-1} suspension of infected brain or limb tissue intraperitoneally. When definitely ill,‡ usually on the 3rd day, brain and limb tissue, the skin being removed from the latter, was collected separately and prepared(7) as antigens. It should be stated here that the titer of virus in limb tissue derived from 11-day-old mice was greater than $10^{-7.5}$, confirming the observations of Gifford and Dalldorf.(2) Extraction of the limb tissue with acetone-ether was found to be more satisfactory than the method of suspension in saline solution followed by centrifugation at 12,000 rpm. For control material, brain and limb tissue was collected from apparently normal Swiss mice of the same age. The complement-fixation test was performed in the manner previously described.(8)

Experimental. Table I illustrates one of the tests. A specific fixation was obtained with both types of Coxsackie virus, used as limb-tissue antigen, without any demonstrable sign of cross reaction. Moreover, antigens made up of brain-tissue virus were not effective. Indeed, following the injection of brain virus into mice, their sera were either negative or of a low titer; whereas after immunization with limb-tissue virus a high titer of fixing antibody was obtained. It would appear that the limb-tissue virus is a highly potent antigen. Finally, no reaction took place between Cox-

5. Olitsky, P. K., and Yager, R. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 719.

6. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.*, 1949, v89, 567.

‡ In experiments with animals deep ether anesthesia was employed.

7. Casals, J., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 339.

8. Casals, J., and Olitsky, P. K., in *Diagnosis of Viral and Rickettsial Infections*, edited by F. L. Horsfall, Jr., Columbia University Press, New York, 1949, 57.

TABLE I.
Showing Complement-fixation Tests with Albany Strains of Coxsackie Virus.

Antigen	Serum					
	Type I brain	Type I limb	Type II brain	Type II limb	Normal	Western equine
Type I limb	0	1/16	0	0	0	0
Type I brain	0	0	0	0	0	0
Type II limb	0	0	1/2	1/32	0	0
Normal limb	0	0	0	0	0	0
Western equine brain	0	0	0	0	0	1/64

Fractions represent the highest dilution of serum showing 2+ or better complement fixation; in the test, serum was diluted 2-fold beginning with 1:2 dilution.

sackie-virus sera or antigens and unrelated virus or apparently normal materials.

Summary. Two Albany strains of Coxsackie virus, Type I (T.T.) and Type II (Fleetwood) were found to be negative for hemagglutination when human O, sheep, chick, and guinea pig erythrocytes were used. On the other hand, the viruses showed distinct positive specific complement fixation without any cross reaction between them. Antigens prepared from infected brain tissue were inactive; those from infected limb tissue were highly active. Tests with human sera and limb-tissue antigens are left for further study.

Received November 4, 1949. P.S.E.B.M., 1949, v72.

The Pituitary-Adrenal Relationship in the Infant Rat. (17525)

JOSEPH W. JAILER*

From the Endocrine Section of the Medical Services and the Chemical Laboratory, The Mount Sinai Hospital, New York.

The administration of adrenocorticotrophic hormone (ACTH) to the rat or guinea pig results in an almost immediate fall in adrenal ascorbic acid content and a more gradual decline in adrenal cholesterol.(1,2) This has been correlated with evidence of adrenal cortical secretion.(3) It had been previously demonstrated by Selye(4) that exposure to stress or the injection of noxious substances causes the secretion of ACTH by the adenohypophysis of the intact animal. Exposure of animals to these same conditions causes a depletion of adrenal ascorbic acid in the intact animal but not in the hypophysectomized animal.(5) The purpose of this investigation was to determine whether the newborn rat could respond to stress and the administration of ACTH in a manner similar to that of the adult.

Infant rats at 2, 4, 6, 8 and 10 days of age were injected with 0.04-0.05 mg of aqueous epinephrine and the adrenals removed one to two hours later. In all, 170 rats from 27 litters were used in this study. Adrenal ascorbic acid was determined by the method of Roe and Kuether.(6) It was found that no decrease in adrenal ascorbic acid occurred until

* Present address, Departments of Medicine and of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University.

1. Sayers, G., Sayers, M. A., Lewis, H. L., and Long, C. N. H., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 238.

2. Long, C. N. H., *Fed. Proc.*, 1947, v6, 461.

3. Sayers, G., and Sayers, M. A., *Endocrinology*, 1947, v40, 265.

4. Selye, H., *Endocrinology*, 1937, v21, 169.

5. Sayers, G., Sayers, M. A., Liang, T. Y., and Long, C. N. H., *Endocrinology*, 1946, v38, 1.

6. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.