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Hemagglutination of Rabbit Erythrocytes by Pus from Cases of Cat Scratch Fever. (25317)

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The possible viral etiology of cat scratch fever, non-bacterial regional lymphadenitis, has been postulated for some time. The demonstration of positive skin tests and complement-fixing antibodies to lygranum in patients with this disease, as well as observation of cytoplasmic, basophilic inclusion bodies similar to those seen in psittacosis, have indicated specifically a virus related to the psittacosis-lymphogranuloma venereum group. Although Mollaret(1) has been able to transmit the disease in humans and monkeys using filtrates of pus from lymph nodes, numerous efforts to isolate an infectious agent, by use of conventional laboratory media, inoculation of common experimental laboratory animals, and use of tissue culture explants, have failed. This paper is a preliminary report on hemagglutination of rabbit red cells by pus aspirated from lymph nodes of clinical cases of cat scratch fever as possibly a viral type of hemagglutination, as well as attempts to demonstrate specific hemagglutinin inhibiting antibody in serum of patients with this disease, and in rabbits immunized with pus.

Materials and methods for hemagglutination of rabbit red cells consisted of pus from lymph nodes of patients clinically diagnosed as cat scratch fever on the basis of a regional non-bacterial lymphadenitis and a history of one or more cat scratches. The pus, as received, was diluted 1:5 in physiological salt solution and 2 of the samples had been heated at 56°C for one hour on consecutive days for preparation of skin-test antigen. This material, either untreated or heated, was serially diluted in phosphate buffered saline through 1:512. One drop of each dilution was then mixed with one drop of a 2% suspension of washed rabbit red cells. The tubes were incubated in water bath at 37°C for 30 minutes, after which they were centrifuged lightly at 1500 rpm for 30 seconds, and observed for hemagglutination by shaking the tubes gently and noting appearance of clumped red cells. Trypsinized red cells were prepared by treating the suspensions with 0.1% trypsin (Difco) for 30 minutes in water bath at 37°C. The preparation of tanned cells consisted of similar treatment with dilute tannic acid. The human sera employed consisted of samples from 2 infected individuals from whom pus was obtained; serum from a person known to have a positive skin test; and serum from an individual suffering from infection with adenovirus. The rabbit sera were obtained before and after immunization of the animal with his own red cells previously treated with pus. Tests for hemagglutinin inhibiting antibody consisted of incubating equal volumes of various dilutions of serum and a dilution of pus containing 4 hemagglutinating units for 30 minutes in water bath at 37°C and then testing for hemagglutination with red cells as described above.

Results. Rabbit erythrocytes were agglutinated by all 10 samples of pus from known

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TABLE I. Titration of Hemagglutinin in 10 Pus Samples.

Sample No.	Skin test	Reciprocal of dilutions 2	4	8	16	32	64	128	256	512
1502	Not known	S	S	S	S	4+	—	—	—	—
1550	Not done	S	S	S	S	2+	—	—	—	—
1263	+	S	S	4	3+	—	—	—	—	—
11-28-1	+	S	S	S	S	4+	—	—	—	—
-2	+	S	S	2+	2+	—	—	—	—	—
-3	+	S	S	4+	4+	—	—	—	—	—
-4	+	S	S	S	S	2+	—	—	—	—
-5	+	S	4+	—	—	—	—	—	—	—
AK-1	—	4+	4+	4+	4+	4+	4+	4+	—	—
XE-1	—	3+	2+	2+	2+	2+	+	+	+	—

S = All cells agglutinated in a single, large clump.

or suspected cases of cat scratch fever including the 2 from skin test negative cases (Table I). Although titers of the material from these latter 2 cases were higher, amount of agglutination in all tubes was not equal to maximal agglutination obtained with samples from skin test positive cases. Samples #1502 and #1550 had been heated to 56°C previous to testing. The same results were obtained with 3 samples using trypsinized and tanned red cells, but human and sheep cells were not agglutinated. Similar tests were negative with purulent lymph node material from 3 cases of lymphadenitis having no clinical or skin test evidence of cat scratch fever.

Table II shows results of tests performed on human and rabbit sera for presence of hemagglutinin inhibiting antibody employing pus from case #1263 diluted so as to contain 4 hemagglutinating units. Only one of the 4 human sera tested (#1263) and the immune rabbit sera demonstrated any antibody.

To confirm specificity of the inhibition, aliquots of serum #1263 and immune rabbit serum were incubated with diluted pus (#1263) and with hemagglutinin negative purulent fluid from a case of lymphadenitis clinically unlike cat scratch fever. The sera were then diluted and retested for antibody with 4 hemagglutinating units of pus as previously described. Inhibiting antibody was absorbed from both sera only by pus from cat scratch fever.

To elute a presumed virus, rabbit cells sensitized with pus were resuspended in saline and incubated at 10°C, 25°C and 37°C for 4 hours. Tests for hemagglutinin in eluates obtained by centrifugation using fresh rabbit cells were negative.

Discussion. Lack of previous evidence of hemagglutination in cat scratch fever and the possibility that the procedures described may be useful in diagnosis of the disease prompted this preliminary report. Good evidence that the hemagglutinin is a virus has been obtained in our laboratory and will be published.

Summary. Hemagglutination of rabbit red cells with pus obtained from lymph nodes of 10 cases of cat scratch fever was demonstrated.

TABLE II. Titration of Hemagglutinin Inhibiting Antibody in Human and Rabbit Sera.

Serum	Clinical diagnosis	Skin test	Reciprocal of serum dilutions 2	4	8	16	32	64	128	256	512	1024	2048
1502	CSF	Not known	S	S	S	S	S	S	S	S	S	S	S
1503	Adenovirus	Neg	S	S	S	S	S	S	S	S	S	S	S
1550	CSF	Not done	S	S	S	S	S	S	S	S	S	S	S
1263	CSF	Pos	—	—	—	—	—	—	S	S	S	S	S
Normal rabbit	—	—	S	S	S	S	S	S	S	S	S	S	S
Immune rabbit	—	—	—	—	—	—	—	—	—	—	3	3	3

S = All cells agglutinated in single, large clump. CSF = Cat scratch fever.
Hemagglutinin control—#1263 pus diluted in saline to contain 4 hemagglutinating units—"S" agglutination.

Specificity of hemagglutinin was indicated by inhibition by serum from a known case of the disease and by rabbit antiserum to the pus.

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Accumulation of *para*-Aminohippurate by Slices of Kidney from Rabbits of Various Ages.* (25318)

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Previous investigations have shown a gradual increase in renal tubular function *in vivo* from fetal life to maturity. Williamson and Hiatt(1) observed rapid increase of phenolsulphonphthalein (PSP) excretion by rabbit kidney from 26th day of gestation to 10th day postnatally when PSP excretion attained adult levels. In the rat(2) ability of tubules to absorb an intravital dye such as trypan blue began at 12 days of age and reached a maximum at 28 days; intravital staining was directly correlated with acquisition of a brush border. Levine and Levine(3) working with fetal, newborn and adult rabbit also report a gradual increase in C_{PSP} until 1 month of age. Experiments on development of renal tubular function *in vitro* cover only the post-natal period. Deyrup(4) demonstrated a low S^{35} uptake in the newborn rat when compared to adult. Rennick(5) observed that transport systems for tetraethylammonium (TEA) and *para*-aminohippurate (PAH) developed at different ages in the puppy: that for PAH at 3-4 weeks of age and that for TEA at 7 weeks of age. This report extends investigation of development of renal tubular function *in vitro* into the fetal period. Accumulation of PAH and chlorphenol red (CPR) by kidney slices is used as a measure of tubular function from fetal life to maturity in the rabbit. Contrary to previous reports(1-5) these data show that instead of a gradual increase in function with age there is less PAH accumulation in the slice from newborn than from either fetus or adult.

Methods. Rabbits of various ages were used: fetuses of 26 to 28 days gestational age; nurslings from 6 hours to 22 days; adult males and females, pregnant and non-pregnant. Essentially methods described by Cross and Taggart(6) were utilized throughout. Slices weighing 200-300 mg(7) were incubated at 30°C for 60 minutes in Krebs-Ringer phosphate(8) with either PAH or chlorphenol red 7.4×10^{-5} M. Control flasks containing 5×10^{-5} M dinitrophenol were included in most experiments. At end of incubation, slices were removed, blotted and placed in 10% trichloroacetic acid for PAH determination. Aliquots of the medium and the filtrate were analyzed for PAH(9). When chlorphenol red was used slices were removed, blotted and smashed on glass slide for direct microscopic visualization. Nitrogen content of slices was determined by micro-Kjeldahl(10); dry weight by drying a minced slice first on steam bath and finally to constant weight over phosphorus pentoxide. Mitochondria were prepared by differential centrifugation of kidney homogenates in 0.25 M sucrose. Their capacity for oxidative phosphorylation (phosphorus/oxygen ratio) was measured(11) using α-ketoglutarate as substrate. Phosphorus was estimated colorimetrically(12) and oxygen consumption determined manometrically.

Results. Accumulation of PAH by slices: On the basis of wet weight, slices of fetal kidney showed greater accumulation of PAH than did those of the neonatal animal. Increased concentration of PAH in the medium (4.4×10^{-4} M) did not alter these results.

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