oxytocic, hypophysiotropic) for vasopressin and CRF may be tentatively related to possibly close structural relationships between the 2 molecules(2,13). Available results do not eliminate the possibility(14) that these large doses of vasopressin may have potentiating effects on circulating ACTH and/or direct effects on the adrenal cortex.

Summary. The CRF preparation *fraction D* stimulates release of ACTH in animals with hypothalamic lesion or pharmacological blockade, at doses where pressor equivalents as pure lysine vasopressin are inactive. Hypophysiotropic activity of fraction D observed *in vitro* is therefore confirmed *in vivo* and should be attributed to a substance different from vasopressin.

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Goat Red Blood Cells in Agglutination Test for Infectious Mononucleosis.* (24849)

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Taiwan is one area of the world in which sheep red blood cells (rbc) are not readily available. Sheep are not economical animals to raise on this island because of high mortality from the liver fluke (*Dicrocoelium dendriticum*). Goats, however, are available. This investigation was undertaken to determine whether goat rbc could be substituted for sheep rbc in agglutination test for infectious mononucleosis(1). The study was designed solely to compare agglutination of goat cells with sheep cells when tested with serum containing heterophile antibody and not to evaluate the clinical significance of these reactions. The ox cell hemolysin test was also performed, since this test had been recommended as a substitute for sheep cell agglutination(2,3), and ox cells can be easily obtained on Taiwan.

Materials and methods. Serums: A total of 161 serums were tested; 61 were from patients and 100 were considered normal controls. Of the patient serums 42 came from the U.S. military dispensary, Taipei and 19 were obtained from the Univ. of Chicago Clinics (through the courtesy of Dr. Ross Benham, Director of the Clinical Microbiology Laboratory). In each case the serum had been submitted for determination of heterophile antibodies, indicating that infectious mononucleosis was being considered in the differential diagnosis. No effort was made to classify these patients by clinical criteria for diagnosis

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TABLE I. Comparison of Sheep and Goat Red Blood Cell Agglutination by 100 Serums from "Normal" Young Adults.

Rbc	<7	7*	14	28	56	112
Sheep	72	19	9	0	0	0
Goat	7	22	37	26	8	0

* Reciprocal of final serum dilution.

of infectious mononucleosis. The 100 serums used as normal controls were obtained from the serological laboratory of Taipei County. They were negative for syphilis and came from young adults. *Heterophile agglutination test*: The differential test of Davidsohn(4) was carried out. In the presumptive test the serum was heat-inactivated only and tested with both sheep and goat rbc. In the differential test aliquots of the serum were absorbed with guinea pig kidney antigen and beef red cell antigen. Each aliquot was then tested with both sheep and goat rbc. *Ox cell hemolysin test*: The method followed was that published by Mikkelsen, *et al.*(3) except that 0.2 ml amounts (rather than 0.5 ml) of serum, complement and ox cells were employed. *Source of rbc*: Sheep (*Ovis aries*) blood, goat (*Capra hircus*) blood and ox (*Bos taurus*) blood were obtained from the Taiwan Serum and Vaccine Laboratory. The only source of sheep rbc on the island is from the sheep maintained in that laboratory for experimental purposes.

Results. The results of testing 100 sera in the presumptive test with sheep and goat rbc to determine the "normal" range of agglutination are shown in Table I. Only 28 of these serums caused agglutination of sheep rbc (at 1:7 and 1:14 dilution) while most of the serums did agglutinate goat rbc in dilutions ranging up to 1:56.

Table II shows the results of testing the 61 serums from patients in the differential agglutination test with both sheep and goat rbc and in the ox cell hemolysin test. The serums are grouped according to their reaction in the presumptive test with sheep rbc. The 27 serums agglutinating sheep rbc in dilutions from 1:56 to 1:3584 in the presumptive test may be considered "positive" since absorption with guinea pig kidney antigen failed to remove the reacting antibody while it was completely removed by beef rbc antigen. These same 27 serums agglutinated goat rbc at dilutions ranging from 1:448 to 1:7168. Again absorption with guinea pig kidney failed to remove the antibody while beef rbc reduced the titer to the range found with the normal serum. Thus, the reaction in the goat cells was similar to that in the sheep cells except for a higher titer which averaged about 4-fold. These 27 serums all reacted in the ox cell hemolysin test. The next 31 serums listed in Table II showed "negative" reactions in the differential test with both sheep and goat rbc. Any antibody measured in the presumptive test (up to 1:28 with sheep rbc and up to 1:112 with goat rbc) was completely removed by guinea pig kidney and frequently not removed by beef rbc.

TABLE II. Results of Tests of 61 Serums from Patients with Possible Infectious Mononucleosis.

	Sheep-rbc-agglutination			Goat-rbc-agglutination			
No. of serum c̄ same sheep rbc titer	Presumptive test	Absorbed c̄ guinea pig kidney	Absorbed c̄ beef rbc	Presumptive test	Absorbed c̄ guinea pig kidney	Absorbed c̄ beef rbc	Ox-cell hemolysin
1	3584	3584	0	3584	3584	0	6144
6	1792	448–1792	0	3584–7168	896–1108	0–56	384–6144
6	896	224– 448	0	896–3584	448–1792	14–28	96– 768
3	448	56– 224	0	1792–3584	896–3584	14–28	192– 768
3	224	28– 112	0	896–1792	224– 896	14–28	48– 384
4	112	28– 112	0	448– 896	56– 448	0–28	48– 192
4	56	28	0	448– 896	112–1792	0–28	24– 48
2	28	0	7	56– 112	0	56	0
5	14	0	0	28– 56	0	14–28	0–nt
7	7	0	0	14– 56	0	7–14	0–nt
17	0	0	0	0– 28	0	0–14	0–nt
*	14, 14, 0	14, 0, 0	0, 0, 0	28, 28, 28	28, 14, 14	0, 0, 0	nt, 0, 0

Results are expressed as the reciprocal of final serum dilution. In agglutination test 0 = <7. In hemolysin test 0 = <6.

* Three serum with unusual low titer reaction patterns. nt = not tested.

Again, agglutination in goat rbc closely paralleled sheep rbc agglutination except that the titer was higher with goat cells. Fifteen of these serums were tested by ox cell hemolysin and none reacted.

The last 3 serums at bottom of Table II showed a low titer reaction different from other negative ones. The titer of antibody in the presumptive test (1:14 with sheep rbc and 1:28 with goat rbc) was too low to be considered positive, but it was not removed by absorption with guinea pig kidney in 1 serum with sheep cells and in all 3 serums with goat cells. It was removed in each case by beef rbc antigen. Two of these serums were tested in the ox cell hemolysin test and failed to react.

Summary and conclusions. Sera from 61 patients showed similar reactions with both sheep and goat rbc in the differential agglutination test for infectious mononucleosis. Twenty-seven sera caused positive differential agglutination with both sheep and goat rbc and reacted in the ox cell hemolysin test. No other sera reacted positively in any of the tests. Goat cells reacted with higher dilution of serum than sheep cells with both patient serums and with 100 normal serums. A positive reaction in goat rbc was of approximately 4-fold higher titer than with sheep rbc. Within limits of these investigations it is concluded that the differential agglutination test with goat rbc and the ox cell hemolysin test gives the same laboratory information for diagnosis of infectious mononucleosis as is obtained from the sheep rbc agglutination test.

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Agglutinating Action of Heat-Inactivated Passage A Mouse Leukemia Filtrates on Mouse Red Blood Cells.* (24850)

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Since a potent mouse leukemia virus (Gross) has been developed by serial cell-free passage through newborn mice of an Ak-leukemia-derived agent(1,2), an attempt was made to determine whether leukemic filtrates containing this agent would agglutinate, or hemolyze, mouse red blood cells (rbc) *in vitro*. In preliminary tests, heated (60°C ½ hr) filtrates were also used, as controls. The surprising observation was made that fresh filtrates were with rare exceptions essentially inactive, whereas heated filtrates had consistently a distinct agglutinating effect on mouse erythrocytes. Experiments were therefore carried out to determine some of the conditions related to agglutinating action of mouse leukemia filtrates on mouse rbc *in vitro*.

Methods. Filtered (Selas 02) extracts of 20% concentration were prepared in usual manner(1-3) from C3H donors with primary, passage A(1,2) virus-induced leukemia. Part of extract was placed in ice-water at 0°C, and used within a few hours for the test. Another part of extract was heated at 55°C for ½ hour. In a few experiments, extracts were also heated to temperatures varying 40° to 60° for ½ hour. Similar extracts were prepared from spontaneous Ak mouse leukemias, from several spontaneous C3H mouse mammary carcinomas, from x-ray induced C3H leukemias, from normal organs (spleen, liver, heart, lungs, kidneys, testicles), and embryos, removed from young healthy C3H mice. *Red blood cells* were obtained by heart puncture,

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