and 17-OHCS levels, they indicate that this effect is transitory. These findings suggest that reserpine is not a specific stimulant of the hypophysis which causes hypersecretion of ACTH, but that the acute ACTH hypersecretion following reserpine administration may be due to the non-specific transitory stress reaction brought about by the drug.

Summary. Acute reserpine treatment of guinea pigs, rats and human patients resulted in activation of the pituitary-adrenal cortex axis demonstrated by hypertrophy of adrenals, adrenal ascorbic acid depletion and higher 17-KS and 17-OHCS levels. These changes disappeared during chronic treatment. It is hence suggested that reserpine through its transistory stimulating effect on the pituitary-adrenal cortex axis acts as a non-specific stimulus, which loses its effect on chronic administration.

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Propagation of Measles Virus in Suspensions of Human and Monkey Leucocytes.* (26409)

ROBERT B. BERG† AND MELVIN S. ROSENTHAL† (Introduced by J. F. Enders)

Research Division of Infectious Diseases, The Children's Hospital Medical Center, and Departments of Pediatrics and Bacteriology and Immunology, Harvard Medical School, Boston

Many viral diseases are associated with a reduction of circulating leucocytes which as a rule is most conspicuous when patients manifest clinical and laboratory evidence of diffuse viral dissemination. The leucopenia accompanying measles is characterized by an absolute reduction of both lymphocytes and polymorphonuclear leucocytes. In attempting to elucidate factors responsible for leucopenia in this disease experiments were designed to determine whether *in vitro* leucocytes of several animal species including man were capable of supporting the growth of measles virus.

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† Postdoctoral Research Fellow, U.S.P.H.S.

Materials and Methods. Preparation and

maintenance of leucocyte suspensions. Fresh venous blood was withdrawn aseptically from healthy human subjects, leukemic patients, a cynomolgus monkey, a domestic rabbit, mongrel dogs and chickens. Fresh cardiac blood was obtained from mice (Webster strain) and guinea pigs. To each 10 ml of blood was added 0.0025 mg of sterile heparin and 2.5 mg of sterile Bacto-Phytohemagglutinin (Difco). Samples were shaken gently by hand for 3-10 minutes and then centrifuged at 300-500 rpm for 3-5 min. at room temperature. Supernatant fluid and buffy coat, containing some red blood cells and the majority of leucocytes were removed and washed 3 times in medium 199(1) and finally resuspended in medium 199 containing 20% calf serum, 50 units of penicillin/ml and 50 μg of streptomycin/ml. When serum of human subjects or animals had been found to be free of measles neutralizing antibodies, heparinized whole blood was usually employed without further treatment as a culture system. Nucleated cell counts of final preparations varied between 5 x 10^6 and 10 x 10^6 cells/ml. Counts were not performed on chicken blood. Aliquots of 1 ml of washed or unwashed blood cell suspensions were delivered to 150 x 16mm test tubes. *Virus.* Three stocks of the Edmonston strain of measles virus were used(2). The first had been passed serially 29 times in human kidney cell cultures and the second 28 times in human kidney cell cultures and once in dog kidney cell culture. The third consisted of the 14th chick embryo cell culture passage of the attenuated Edmonston strain(3). These stocks will be referred to, respectively, as HK virus, DK virus and attenuated virus. *Viral Assay.* Infectivity titers were determined in cultures of a continuous line of human amnion cells designated WS in this laboratory(5). Serial ten-fold dilutions of leucocyte suspensions previously inoculated with measles virus were prepared in cold bovine amniotic fluid medium and immediately 0.1 ml aliquots of each dilution were added to each of 3 WS cell cultures. Cultures were kept stationary at 37°C and observed for formation of typical giant cells on the 5th, 10th and 14th days.

WS cells were prepared and nourished in Eagle's basal medium(6,7) modified as follows: $MgSO_4$ (0.1 g/1) was substituted for $MgCl_2 \cdot 6H_2O$ (0.17 g/1), cystine was increased to 24.03 mg/1, methionine was decreased to 7.46 mg/1; Inositol (0.4 mg/1) (8) and supplemental arginine (17.42 mg/1) were added. Tissue culture fluids were changed on the 5th and 10th days after inoculation and cultures were discarded after 2 weeks.

Experimental. Multiplication of measles virus in human white cells was demonstrated in the following experiment. Each of 2 tubes containing 1 ml of washed human blood cell suspension was inoculated with 0.1 ml of a 10-fold dilution of stock HK virus and closed with solid stoppers (West Co., Phoenixville, Pa.—white, non-toxic). Undiluted stock HK virus had an infectivity titer of 3.8 log 10 TCD_{50}/0.1 ml. After these suspensions had incubated at 37°C for 4 days in a roller wheel turning at about 18 rph, 0.1 ml aliquots were passed to freshly prepared washed blood cell cultures. Third and fourth passages were made at 4 day intervals. Aliquots representing a dilution of the original stock HK virus of 10^5 times were taken from the final passage on the 4th, 7th and 14th days and titrated for infectivity. Titers were recorded respectively as 5.3, 4.8 and 1.8 log 10 TCD_{50}/0.1 ml.

In contrast, when tubes containing 1 ml of medium 199 plus 20% calf serum but no leucocytes were inoculated with 0.1 ml of undiluted HK virus and later assayed for infectivity, virus could not be demonstrated after 2 days incubation. Tubes containing human serum free of measles antibody were similarly inoculated. From these only 0.75 log 10 TCD_{50}/0.1 ml of HK virus was recovered after 2 days and none after 7 days. Suspensions of red blood cells and of leucocytes which had been rapidly frozen and thawed also failed to support survival or multiplication of measles virus.

In suspensions of both washed and unwashed leucocytes multiplication of virus was demonstrated during a single passage. In each experiment the pooled contents of 2 or

TABLE I. Maximal Infectivity Titers Found in Blood Cell Suspensions after Inoculation with Measles Virus.

Exp. No.	Source of blood cell suspensions	Virus	Titers (log 10 TCD_{50}/0.1 ml) of suspensions, days after inoculation* 0 day	4 days	7 days	14 days
1	Healthy human	HK	1.8	2.45	3.50	NA†
2	" "	"	1.8	1.75	3.50	NA
3	" "	"	1.8	4.75	4.75	0
4	" "	"	1.8	4.25	4.50	1.75
5	" "	"	1.8	3.50	4.25	0
6	" "	"	1.8	2.75	3.75	0
7	" "	"	2.8	3.00	4.25	2.5
8	" "	"	2.8	2.66	2.50	NA
9	" "	Attenuated	.8	2.25	2.75	0
10	" "	"	.8	3.25	2.50	0
11	Leukemic patient	HK	1.8	4.75	3.75	0
12	" "	"	1.8	2.75	2.75	NA
13	Cynomolgus monkey	"	1.8	3.25	3.50	0

* Titers of the stock HK and attenuated viruses were 3.8 and 2.8 log 10 TCD_{50}/0.1 ml. Zero day titer represents the calculated infectivity titer of the suspension immediately after inoculation with diluted stock virus. All titers were determined in WS cells.

† Not assayed.

more stock virus ampoules were employed as inocula and at least 2 blood cell suspensions were inoculated with 0.1 ml, appropriately diluted in bovine amniotic fluid medium and maintained at 37°C in a roller wheel. Aliquots of blood cell suspensions were pooled from 2 or more tubes and infectivity titers determined on the 4th, 7th and 14th days after inoculation. Early experiments showed that infectivity titers were maximal on the 4th to 7th days. Only rarely could virus be detected after 3 weeks. Results of these experiments are presented in Table I (Exp. 1-10).

Experiments were performed to determine whether in suspensions of human leukemic and certain animal leucocytes multiplication of measles virus occurred. Samples of blood were withdrawn from 2 children ill with acute leukemia. One child had a differential white cell count of 96% blast forms, 3% lymphocytes and 1% polymorphonuclear leucocytes. The other child's count showed 99% blast forms and 1% lymphocytes. The immature cells of both patients were considered likely to be lymphoblasts by the Tumor Therapy Service at The Children's Hospital Medical Center. HK virus, the only virus tested, multiplied in suspensions of wbc from these children (Table I, Exp. 11-12).

Attempts were made to propagate measles virus in leucocytes obtained from 6 different animal species. At least 2 separate specimens of blood were drawn from each animal. It was necessary to pool the cardiac blood of 6 mice for each experiment requiring the use of mouse blood. When mouse or chicken blood was used, the cells were not washed. In the case of all other animals both washed and unwashed blood cells were employed. Leucocytes obtained from chickens, dogs, rabbits, mice and guinea pigs did not support the multiplication or survival of HK virus. The single monkey used had been immunized with attenuated measles vaccine(8) and therefore all samples of its blood were washed. On 2 occasions HK virus failed to multiply in monkey leucocytes, but in one experiment propagation of the virus was demonstrated (Table I, Exp. 13). It was postulated that DK virus, having been adapted to dog kidney tissue culture, might multiply in canine leucocytes and that attenuated virus, having been adapted to chick embryo tissue culture, might multiply in chicken leucocytes. Canine suspensions were inoculated with 310 TCD_{50}/0.1 ml of attenuated virus, and avian suspensions with 63 TCD_{50}/0.1 ml of attenuated virus. In neither was evidence of multiplication obtained.

Preliminary observations have shown no significant difference in the rates of disappearance of particular cell types or the drop in total white cell counts in inoculated as

compared with uninoculated human leucocyte cultures. In both, virtually all polymorphonuclear leucocytes disappeared by the 4th day of incubation. Microscopic examination of infected and non-infected leucocytes stained with Wright's and acridine orange (9) stains failed to reveal distinctive morphologic variations. Efforts were made to localize the site of intracellular virus by means of the indirect fluorescent antibody technique (10). To date the presence of non-specific staining material has made it difficult to interpret the results.

Discussion. Occurrence of viremia in measles was first established in 1905 by transmission of the disease to susceptible subjects with whole blood(11) derived from patients. Measles virus has been isolated in tissue cultures from the blood of humans and monkeys in this laboratory(2,12). Papp transmitted the infection to children using suspensions of washed leucocytes obtained from measles patients(13). Peebles isolated the virus in tissue cultures from the buffy coat of typical cases(14). In this study we have shown that propagation of the virus is supported by suspensions of human and simian leucocytes. Taken as a whole these observations suggest that the virus during the viremic stage is closely associated with the blood and in particular with the blood leucocytes.

Earlier researches demonstrated that certain viruses of animal origin could be propagated in cultures of rabbit, porcine and avian leucocytes(15,16,17,18) after multiplication of the cells had taken place. Moreover, as we have now found with measles virus and primate cells, Dunne and his co-workers observed that Newcastle Disease virus multiplies in suspensions of porcine blood leucocytes. Although no direct evidence as yet has been obtained for multiplication *in vivo* of viruses in circulating leucocytes, these findings *in vitro* strongly suggest that it occurs in these elements. If so, as Dunne has postulated, one factor underlying the leucopenia can be visualized, since infection of the susceptible cell usually terminates in its destruction. Infection and death of immature members of the leucocytic series may, however, also play a significant role. Supporting data are not available in the case of measles and many other viral infections, but in feline panleucopenia(19) nearly all the cellular elements of the bone marrow appear to be attacked and destroyed by the responsible virus.

Our attempts to distinguish infected from uninfected cells morphologically and by means of immunofluorescence staining have so far proved unsuccessful. It therefore remains to be determined which of the leucocytic series are capable of supporting viral multiplication. Dunne and co-workers noted that in porcine cultures, polymorphonuclear leucocytes had essentially disappeared during the first 24 hours of incubation. Accordingly, they concluded that hog cholera virus multiplied in the remaining mononuclear cells. The data from our serial differential counts also suggest that measles virus reproduces itself in mononuclear cells. These data are supported by the growth of the agent in leukemic blood essentially devoid of polymorphonuclear cells. Moreover, inoculated normal white blood cell suspensions yielded the highest concentration of virus between the 4th and 7th days of incubation when a negligible number of polymorphonuclear leucocytes were present. Extracellular virus possibly derived from polymorphonuclear cells during the first 3 days of incubation would have been largely inactivated at this time. Thus, although viral multiplication appears to occur chiefly in mononuclear cells, the possibility that it also takes place to a certain extent in polymorphonuclear cells cannot at present be excluded.

The data now available do not indicate whether viral multiplication occurs in leucocytes originally present in the blood specimens or only in descendents of these cells. In view, however, of the rapidity of increase and decline of virus and the slow increase in mononuclear leucocytes observed in plasma roller tube cultures(18) it seems probable that the agent multiplies, at least in part, in surviving cells originally present in the circulating blood of the donor. Therefore, in this communication we have referred to the blood

cell preparations as "suspensions" rather than "cultures."

Summary. Multiplication of measles virus has been demonstrated in suspensions of human and simian blood leucocytes. Under the experimental conditions employed the virus failed to multiply in canine, rabbit, guinea pig, mouse and chicken blood leucocytes. Evidence is presented which indicates that multiplication takes place in mononuclear cells.

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Response of Rats of Various Ages to Erythropoietin.* (26410)

JOSEPH F. GARCIA AND DONALD C. VAN DYKE

Donner Laboratory of Medical Physics, Lawrence Radiation Laboratory, University of California, Berkeley

New-born rats develop an anemia soon after birth and it becomes most severe when the animals are 15-20 days of age(1,2). In the rat this is the period of maximum growth rate of the body as a whole as well as the period of maximum growth rate of total circulating red cell volume. During the time that the anemia is developing, iron kinetics studies reveal that erythropoiesis is proceeding more rapidly than at any other time in the life span of the animal(3). From these observations it has been suggested that the anemia in the rat results from the disproportionate growth of the body in relation to the ability of the marrow to supply erythrocytes. This implies that the marrow is functioning normally and at maximum capacity during the anemic period. It has been shown that rats do not show an erythropoietic response to hypoxia during the neonatal period(4).

It was considered of interest to determine whether erythropoietin, which is active in adult rats, would stimulate erythropoiesis during the period of neonatal anemia.

Materials and methods. Both total circulating red cell volume measurements and Fe^{59} red cell incorporation studies were used to judge the erythropoietic response to erythropoietin. Groups of normal male rats of the Long-Evans strain of 12, 23, 87, 230, and 470 days of age were used to determine the effect of erythropoietin on total circulating red cell volume. Control and experimental groups, equal in number and of the same average body weight at the beginning of experiment, were studied at each age period. Half

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