

and death.

Cotton rats inoculated intranasally or subcutaneously with a 10^{-3} dilution of virus became weak and died within 48 to 72 hours after inoculation, while guinea pigs displayed a febrile response only and survived subcutaneous inoculation.

The lesions in inoculated mice consisted of glial nodules scattered through the corpus callosum, internal capsule, thalamus and mid-brain. There was almost complete destruction of the Purkinje cells of the cerebellum. The cerebral cortex was essentially free of lesions. The lumbar portions of the spinal cord showed an occasional necrotic neuron in the ventral gray horn. Demyelination was present in certain fiber tracts and nerve trunks. The heart showed interstitial round cell infiltration and mild fiber fragmentation in the auricular myocardium. The spleen, lungs, liver, gut,

and kidneys were free of demonstrable lesions.

Summary. During the past 3 years encephalomyocarditis virus was isolated twice from captive primates imported to Florida and once from a wild squirrel originating in Florida.

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Effect of Temperature on Activity of Guinea Pig Complement. (22195)

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When human complement (hu C') and sensitized sheep erythrocytes (EA) interact, both extent of lysis and shape of the lytic curves obtained are markedly dependent on temperature(1). These findings have recently been explained(2) on the basis of proportionality between temperature and rates of formation and decay of an intermediate complex EAhuC'_A.

Since the great majority of both experimental and routine laboratories have used guinea pig (g.p.) rather than hu C' in their investigations, and since the choice of 37°C as reaction temperature appeared to be quite arbitrary, the effect of temperature on lysis by g.p.C' was investigated. These studies were greatly facilitated by data from several laboratories(3,4) on EA_{g.p.C'}1,4,2, the complex between EA and g.p.C'1, C'4 and C'2.

Methods. Sheep blood in 500 ml lots was preserved with the addition of 2000000 units

of penicillin "G" and 25 ml of 0.15 M Trisodium ethylenediamine tetraacetic acid (Na₃E.D.T.A.)(4). Veronal buffer(5) containing 5×10^{-4} M Mg⁺⁺ and 1.5×10^{-4} M Ca⁺⁺ was used for all dilutions and washings. Pooled guinea pig sera, stored in small lots at -25°C, were the source of g.p.C'. Kinetic analyses were carried out according to the technic of Mayer and Levine(6). Bath temperatures were held constant to $\pm 0.1^\circ\text{C}$. All solutions were equilibrated at the temperature of the experiment before mixing.

Results. Two flasks, each containing 10 ml of EA, 10 ml of buffer and 5 ml of g.p.C'/170 were incubated at 37°C, and 27°C respectively. After sampling was completed, 2 more flasks were run at 37°C and 32°C. The reaction curves are plotted in Fig. 1. The extent of reaction at 37°C was considerably less than at either 27°C or 32°C.

The next experiment was designed to study

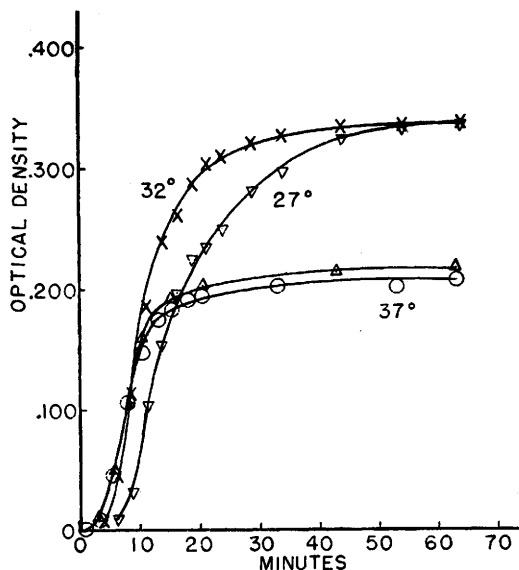


FIG. 1. Effect of temperature on lysis by a constant volume of g.p.C'.

the effect of temperature on formation of the intermediate complex EAg.p.C'1,4,2. The complex was prepared by the method of Leon *et al.*(4), using a sublytic dilution of g.p.C'. Flasks containing 30 ml of cells, 30 ml of buffer and 15 ml of g.p.C'/540 were incubated at 37°C, and 27°C and later the same day at 37°C and 32°C. At intervals 3 ml samples were withdrawn, mixed with 0.5 ml of g.p.C'/

40 and 0.2 ml of 0.15 *M* Na₃E.D.T.A., stoppered and incubated at 32°C for one hour. Na₃E.D.T.A. binds Ca⁺⁺ and Mg⁺⁺ and therefore prevents further reaction of any complement components except for C'3(4,7). The C'3 present in the g.p.C'/40 will lyse only those cells that have reached the stage EAg.p.C'1,4,2, through reaction of EA with g.p.C'/540. The results are plotted in Fig. 2.

Discussion. In the presence of a constant amount of C'3 the extent of lysis is proportional to the quantity of EAg.p.C'1,4,2 available(4,8). The quantity of EAg.p.C'1,4,2 available will, in turn, be a function of the quantity of C'1, C'4 and C'2 present and of the reaction temperature (Fig. 2). Thus, at 27°C and 32°C, where the concentration of EAg.p.C'1,4,2 is *maintained* at a high level for a considerable length of time, more lysis is obtained than at 37°C where the concentration of intermediate is smaller and its stability lower.

In this laboratory, where both hu C' and g.p.C' are used, the majority of lytic reactions are now carried out at 32°C, the temperature previously shown to be optimal for hu C'(1).

Summary. Guinea pig complement is considerably more reactive at 27°C and 32°C than at 37°C. These findings have been ex-

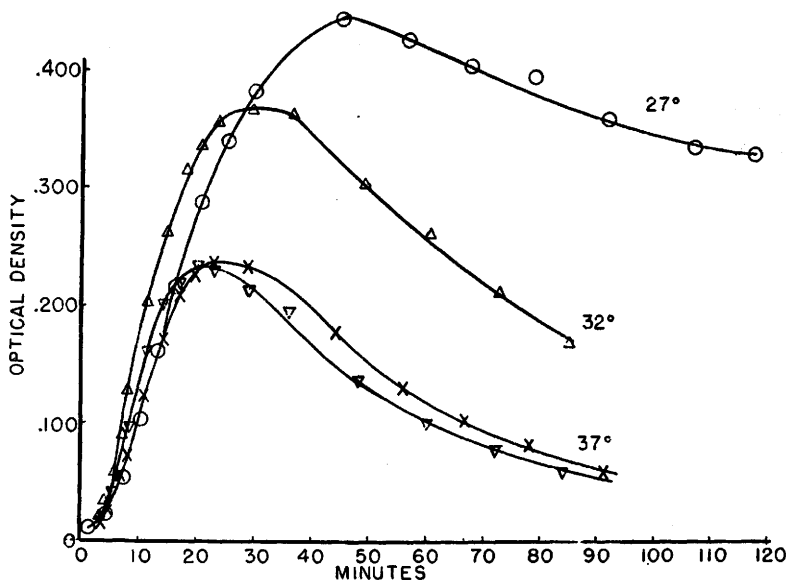


FIG. 2. Effect of temperature on formation and decay of EAg.p.C'1,4,2.

plained by differences in the maximal formation and the stability of an intermediate complex, EAg.p.C'1,4,2.

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Effect of X-Irradiation and Hypophysectomy on Globule Leucocytes In Rat Intestine.* (22196)

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Globule leucocytes are distributed widely in urinary(1), respiratory(2) and digestive mucous membranes (Fig. 1) of the rat. They are distinguished by presence in their cytoplasm of abundant, coarse, metachromatic acidophilic inclusions and by the fact that they occur only in the epithelium and tunica propria. Cytological evidence indicates that they arise by direct transformation from lymphocytes(3). Like lymphocytes, they are reduced in number by treatment with corticotropin or cortisone(2). Because of these ob-

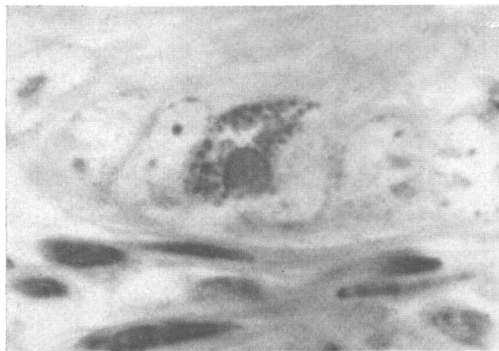


FIG. 1. Globule leucocyte in epithelium of duodenal crypt of hypophysectomized rat. $\times 1450$.

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servations and the well-known radiosensitivity of lymphocytes, this study was made of the response of globule leucocytes to total body x-irradiation. Observations are reported also on effect of hypophysectomy.

Procedure. Female Sprague-Dawley rats weighing 150-190 g were used. During early experiments, control rats were pair fed against irradiated animals. Since it was observed that reduction in food intake was without effect, the animals were fed *ad libitum* in the final experiments. Diet consisted of Purina Laboratory Chow supplemented twice weekly with greens, oranges, and a vitamin concentrate. Temperature of animal quarters was maintained at $75 \pm 3^\circ\text{F}$. All irradiated animals (except as noted below with relation to shielding experiments) were exposed to whole body irradiation while held in flat cylindrical holder constructed of $\frac{1}{4}$ -inch sheet Lucite and radially divided into 9 equal compartments. Radiation was administered by Keleket Theron X-ray therapy machine operated at 200 KV with $\frac{1}{2}$ mm Cu and 1 mm Al filtration. Distance from center line of x-ray tube to table on which animal container rested was 110 cm. Dose rate was indicated by Victoreen rate meter and was held constant at about 11 r/min by varying x-ray tube cur-