Table 1. Temperatures recorded in rabbits inoculated with rinderpest virus.

Time after injection (hr)	Temperature (°F)	
	Rabbit 11	Rabbit 12
24	102.5	102.8
32	103.3	104.5
48	105.6	106.5
56	106.0	107.0
72	102.8	103.9
80	103.0	103.2
96	102.4	103.1

nate transfers, it seems likely that earlier passage membranes could be used with equal success. Titrations of fourth- and ninth-passage membranes have shown the virus to be present in considerable amounts (*2*). No doubt, if a sufficient number of serial passages were carried out in the rabbit, the virus would eventually become attenuated for the calf (*3*).

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3. This work was a joint project of the Grosse Ile Experimental Station, Defence Research Board of Canada, and the Animal Pathology Division, Canada Department of Agriculture, Animal Disease Research Institute, Hull, Quebec. It was conducted under the direction of C. A. Mitchell, director of projects.

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Enzymatic Synthesis of Adenyl-oxyluciferin

In the activation of amino acids, acetic acid, and firefly luciferin by adenosine triphosphate (ATP), pyrophosphate and the corresponding acyl-adenylates are presumably formed (*1*). The synthetic adenylates in all three cases are biologically active. Unfortunately, the enzymatic synthesis of these acyl-adenylates has not been demonstrated. Presumably this is due to the tight binding of the adenylates to the enzyme. Recently we have been able to measure the equilibrium constant for the association of adenyl-oxyluciferin (L-AMP) and firefly luciferase. As expected, it was found that the equilibrium constant of the reaction

$$\text{L-AMP} + \text{E} \rightleftarrows \text{E-L-AMP}$$

was approximately 2×10^9. Apparently a similar tight binding with enzyme exists with regard to the amino acid and acetic acid adenylates. If this is true, it is not surprising that it has been difficult to demonstrate the enzymatic formation of the adenylates of these compounds. However, the intense fluorescence of oxyluciferin makes it possible to measure the disappearance of quantities which are less than the amount of enzyme added. Because of this sensitivity of the fluorescence technique, we have been able to measure the enzymatic formation of L-AMP in the following reaction:

$$\text{ATP} + \text{L} + \text{E} \rightleftarrows \text{E-L-AMP} + \text{POP} \quad (1)$$

The measurement of L-AMP synthesis depends on the following differences in properties between chemically synthesized L-AMP and oxyluciferin: (i) L-AMP has a fluorescence intensity which is only 2 percent of the intensity of oxyluciferin. (ii) At a *p*H of 3 oxyluciferin is extractable into ethyl acetate, whereas L-AMP is not. The solubility of the oxyluciferin in ethyl acetate depends upon the dissociation of the carboxyl group. (iii) When paper chromatography and the solvent system described in Table 1 are used, L-AMP has an R_f of 0.55, while oxyluciferin has an R_f of 0.40.

The results of an experiment in which oxyluciferin, luciferase, $MgSO_4$ and ATP were incubated at *p*H 7.1 are shown in Table 1. The reaction was initiated with ATP. Following the addition of ATP there was an immediate decrease in fluorescence corresponding to the disappearance of oxyluciferin approximately equal to the amount of luciferase present. When the reaction mixture was acidified and extracted with ethyl acetate, the excess oxyluciferin was removed from the reaction mixture. The nonextractable, weakly fluorescent material in the aqueous phase had an R_f of 0.55 which is identical to that of synthetic adenyloxyluciferin. Alkaline hydrolysis gives an increase in the fluorescent intensity and a product with an R_f of 0.4. This, and other evidence which has been recently published, indicates clearly that the material in the aqueous phase is adenyl-oxyluciferin (*2*).

By fluorescence measurements, it has been possible to determine equilibrium concentration of oxyluciferin from which the equilibrium constant for reaction 1 can be calculated. It should be noted that in this reaction the enzyme must be considered in the equilibrium determination. Measurements made on the forward and reverse reactions both give a value of K approximately equal to 2.0×10^5. By using this constant, along with the dissociation constant for luciferase-L-AMP complex, the free energy of hydrolysis of L-AMP can be calculated. If it is assumed that the free energy of hydrolysis of the second phosphate bond in ATP is the same as for the terminal phosphate bond (*3*), the free energy of hydrolysis is obtained by adding the following reactions:

Table 1. Results. The reaction mixture contained the following: oxyluciferin, 3.2 mμmole; $MgSO_4$, 10 μmole; luciferase, 2.0 mμmole; ATP, 4 μmole; brought to final volume of 2.0 ml with 0.1*M* tris-maleate buffer, *p*H 7.1.

Reaction mixture	Fluorescence* intensity	R_f
− ATP	80	0.40
Complete	30	0.55†

* Arbitrary units; excitation, 360 mμ; emission, 540 mμ.
† After ethyl acetate extraction; paper Whatman No. 3-MM; solvent, 30:70 (by volume) mixture of 1*M* ammonium acetate and 95 percent ethanol, *p*H 7.5.

	$\Delta F°'$(kcal)
$\text{ATP} + \text{L} + \text{E} \rightleftarrows \text{E-L-AMP} + \text{POP}$	− 7.2
$\text{E-L-AMP} \rightleftarrows \text{E} + \text{L-AMP}$	+ 12.6
net $\text{ATP} + \text{L} \rightleftarrows \text{L-AMP} + \text{POP}$	+ 5.4
$\text{POP} + \text{AMP} \rightleftarrows \text{ATP} + \text{H}_2\text{O}$	+ 7.7
net $\text{L} + \text{AMP} \rightleftarrows \text{L-AMP} + \text{H}_2\text{O}$	+ 13.1

These calculations were made on the assumption that Mg^{++} has no appreciable effect on the equilibrium of the activation reaction. This point is now being investigated. It is not surprising that the free energy of hydrolysis of L-AMP is considerably higher than that of ATP, since coenzyme A reacts readily with L-AMP to produce AMP and oxyluciferyl-coenzyme A.

The biological significance of the tight binding of acyl adenylates to the enzyme is not clear. However, it should be pointed out that the present evidence indicates that such tight binding restricts the reactivity of the adenylates. For example, L-AMP is capable of reacting with SH compounds such as coenzyme A and cysteine to form the corresponding oxyluciferin derivatives and adenylic acid. However, cysteine is capable of reacting only nonenzymatically with L-AMP, whereas coenzyme A can react with L-AMP only when it is bound to luciferase (*4*).

Note added in proof: M. Karasek *et al.* have recently demonstrated the enzymatic synthesis of tryptophan-AMP (*5*).

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10 March 1958

Test for Genetic Recombination in Kappa Particles of Paramecium aurelia, Variety 4

Kappa particles are microscopically visible structures, about a micron in length, which are distributed at random in the endoplasm of certain strains of paramecia. Furthermore, these particles, numbering several hundred per paramecium, are self-reproducing and mutable, and they contain deoxyribonucleic acid and are capable of infecting other paramecia under certain conditions (*1*). It is clear that the kappa particle shows many properties of a microorganism; hence, another property, genetic recombination, was also looked for.

At least two pairs of characters which do not lie at the same genetic locus must be studied to detect recombination. With kappa particles two such pairs are (i) ability, or lack of it, to produce the poison paramecin and (ii) ability, or lack of it, to persist in paramecia under certain culture conditions. The reason for assuming that these characters are not at the same locus is that they mutate independently of one another. Specifically, *slow hump kappa* particles (*2*) produce paramecin but are lost from animals grown at maximal fission rate at 31°C; *pi* particles (*3*) do not produce paramecin and are not lost under the growth conditions just described. Both types are mutants of the *normal hump kappa* particles found in stock 51 of *Paramecium aurelia,* variety 4. Normal hump kappa particles, like slow hump kappa, produce paramecin, but, like pi are not lost from well-fed paramecia grown at 31°C.

The test for recombination is to determine whether, following placement of slow hump kappa and pi particles in a common cytoplasm, there will appear particles capable both of producing paramecin and of persisting in host organisms grown at maximal rate at 31°C. That is, will normal hump kappa-like particles appear? This test was made as follows. Three types of paramecia, containing pi and slow hump kappa particles, containing only slow hump kappa particles, and containing only normal hump kappa particles, were all grown under conditions which would result in the marked decrease or loss of slow hump kappa particles, as indicated by a marked decrease or loss of poison production. Then the progeny of all three sets were tested for production of paramecin. The animals containing pi and slow hump kappa particles gave results comparable to those given by animals containing only slow hump kappa, both groups showing little or no paramecin production, whereas the normal hump kappa animals produced large amounts of the poison.

If the animals containing both pi and slow hump kappa particles had shown paramecin production similar to that of the normal hump kappa animals, then appearance of a recombinant type of particle in the former would be a possible explanation. In the absence of such a finding, the simplest conclusion is that under the conditions of this experiment no recombination occurs. This experiment assumed that the recombinant would behave as normal hump kappa do in the presence of pi—that is it would become abundant in each organism (*4*). However, it is possible that the recombinant might be of a new type which would exist only in a very low frequency. The experiment, as executed, does not test this possibility (*5*).

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5. This article is contribution No. 647 from the Zoology Department of Indiana University. During the course of this investigation, I was a predoctoral fellow of the National Science Foundation. This work was aided in part by a grant from the Rockefeller Foundation for work in genetics at Indiana University, and in part by a grant-in-aid to Professor T. M. Sonneborn from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

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Acetylcholine Hydrolysis in Psychiatric Patients

In a recent report (*1*) I suggested the possibility that the mechanism underlying the relationship between scotopic threshold changes and psychiatric disorders may be a deficiency in the acetylcholine-cholinesterase balance.

Granger (*2*) reported that schizophrenics manifested a significant elevation of the dark-adaptation curve along the log-luminance axis, without alteration of shape, as compared with normals. Rubin and Goldberg (*3*) employed Sarin (isopropyl methylphosphonofluoridate), an anticholinesterase, in experiments designed to determine the effect on scotopic visibility of measured quantities of inhaled material and found significant elevations of the absolute visual threshold. A second study (*4*) indicated that conjunctival instillation had no significant effect on dark adaption, while significant elevation of the dark-adaption threshold did occur when both eyes were protected from the atmosphere containing Sarin and in both eyes although only one had been protected. Rubin *et al.* (*5*) then performed another test of the hypothesis that the eventuation of the threshold response was localized in the central nervous system. Following exposure to Sarin, it was found that the tertiary atropine salt (atropine sulfate) reduced the elevated threshold to the preexposure level while the quaternary methosalt of atropine (atropine methyl nitrate) had no effect on the Sarin-produced elevated threshold. The ability of the tertiary salt to pass the blood-brain barrier while the quaternary salt does not suggested that the cholinergic blocking effect observed was acting centrally. It was further suggested that acetylcholine-cholinesterase concentrations are important determinants of the dark-adaption process. These results suggested that the elevated dark-adaptation thresholds observed in schizophrenia could be attributed to some biochemical imbalance between the enzyme and its substrate.

With this paradigm in mind, it was posited that the hydrolysis rate of acetylcholine by erythrocyte cholinesterase should be different for normals and for patients admitted to a psychiatric institute (*6*). Blood samples (5 ml) were obtained from 10 volunteers among the hospital staff and from 23 patients within 24 hours after admission and prior to the administration of any kind of therapy. Michel's electrometric method (*7*) was employed to determine the activity of the cholinesterase (*8*). The rate of hydrolysis of acetylcholine by erythrocyte cholinesterase for the normals and institutionalized patients is presented in Table 1. The standard error of regression for the normals was found to be $s_b = \pm 0.0003$, and the fiducial limits of the slope at the 5-percent level of confidence for $(n\text{-}2)df$ was found to be $l_1 = -0.0106$ and $l_2 = -0.0088$. The slope for each patient was calculated and then evaluated relative to the fiducial limits calculated for the normal sample. Six out of 23 patients had slopes that fell within the fiducial limits of the sample, while 17 distributed themselves into two groups that differed significantly from the linear regression that characterized the sample of normals. Ten patients showed significantly slower, and seven patients showed significantly faster, hydrolysis of acetylcholine by erythrocyte cholinesterase. Of the six patients whose slopes were in the normal hy-