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The Effect of Altering Metabolic Rate on the Mortality and Incubation Time of Rabies* (32884)

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(Introduction by S. A. Peoples)

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The basis of the postexposure treatment of rabies, except for the actual elimination of the virus from the wound, depends upon the length of the incubation period; for if this is long enough, the patient can be protected by a combination of passive and active immunization. From this viewpoint, it would be significant if certain procedures would lengthen the incubation period and still allow the host to actively participate in his own defense.

The incubation period of rabies in bats may be prolonged by placing this host in low ambient temperatures and conversely may be shortened in higher than normal temperatures (1). It has been shown that the metabolic rate of bats varies directly with the ambient temperature (2). Subsequent reports (3,4) have amply confirmed this relationship. It is not known whether the incubation times for rabies in bats placed in different ambient temperatures is due to changes in metabolic rate or some other factor associated with changes in temperature.

This paper reports the results of a series of experiments designed to evaluate the effect of altering the metabolic rate of homothermic mammalian hosts on the incubation time and survival ratio of rabies.

Materials and Methods. Swiss mice of the Webster strain, Sprague-Dawley rats, golden Syrian hamsters and Duncan-Hartley guinea pigs were used in these experiments. To increase the metabolic rate of these animals,

iodinated casein (CI) was added at a 0.1% concentration by weight in their feed. This concentration in the daily diet was sufficient to maintain an increased metabolic rate, without toxic effects, over a period of 30 days. The animals did not show a marked increase in metabolic rate until they had been on the diet approximately 5 days. The feeding of CI was usually started on the same day the virus was inoculated; however, in Expt. 15, the diet was started 10 days before the rats were inoculated so that they would have an increased metabolic rate when they were inoculated with the rabies virus.

To lower the metabolic rate of the animals, propylthiouracil (PTU) was added to the diet in an 0.1–1% concentration by weight. While this drug was tolerated in concentrations up to 4%, no difference in the metabolic rate was found in the higher dosages and palatability problems were encountered at dosages over 2%. The animals' metabolic rate was not noticeably lowered until PTU was given daily in the diet for approximately 10 days. In Expts. 1, 6, and 18, the PTU was given 10–15 days before inoculation. In the rest of the experiments, PTU was started the same day the animals were inoculated with the rabies virus.

Guinea pigs, hamsters, and rats were thyroidectomized at least 14 days prior to virus inoculation in order to compare the metabolic rates and incubation times with respect to rabies in these animals with those that were fed propylthiouracil. Rats were hypophysectomized to reduce the thyroid activity indirectly,

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and thereby lower the metabolic rate by a different mechanism.

The oxygen consumption rate was measured in a closed circuit apparatus in which CO₂ was taken up by soda lime. This method measures the oxygen consumption of the animal at rest, and is reported as ml of oxygen/kg^{3/4} per min according to the method of Kleiber (5). By this method, it was possible to compare the metabolic rates of animals which were receiving metabolically active drugs with the untreated controls. To avoid handling of rabid animals, it was assumed that the uninoculated groups receiving drugs in their diet had the same relative change in metabolic rate as the rabies virus-inoculated animals receiving the same drug at the same time. The oxygen consumption per kilogram^{3/4} per minute was obtained from each of four animals (six when mice were being tested) receiving the same dose of the drug as the rabies inoculated animals and from an equal number of animals not receiving the drug. The oxygen consumption measurement was repeated three times for each animal and the average value used. Following this, the oxygen consumption per kilogram^{3/4} per minute for all the animals in each group were averaged and the normal and treated groups compared. Oxygen consumption per kilogram^{3/4} per minute values were obtained from the treated and untreated groups on the same day two to three times each week for a period of 4 weeks. For the purpose of this study, the metabolic rate was expressed as the percentage difference that the treated group varied from the normal group over the 4-week period.

The strain of rabies virus used was isolated from a Mexican freetail bat (*Tadarida mexicana*) in this laboratory in 1954 (6). The characteristics of this strain of virus are much like those of Virus Fixé. It has a short and uniform incubation period of from 8 to 10 days in mice and rarely induces formation of Negri bodies. Before being maintained by mouse passage, it was passed twice through the freetail bats (*T. mexicana*) and five times through pallid bats (*Antrozous pallidus*). Pools of brain tissue made from the second, third, and fourth mouse passage were used throughout these experiments. Twenty

percent suspensions of mouse brain tissue, containing the virus, in a diluent of sterile skim milk, with 200 units of penicillin and 250 mg of streptomycin/ml, were divided into equal portions, and stored at -70°C. A new vial was used for each experiment. The titer of each pool was determined by both intracranial and intramuscular routes of inoculation for each species of animal to be used experimentally. For these experiments the rabies virus was inoculated intramuscularly into the thigh; 0.1 ml for mice and hamsters, and 0.2 ml for guinea pigs and rats. Dosages used in the experiment varied between 1 and 100 LD₅₀ by this route of inoculation.

Results. Table I is a summary of four experiments with mice, one with hamsters, and two with rats, which received iodinated casein in the diet. Metabolic rates were obtained in experiments 9, 14, 11, 12, and 15. The metabolic rate was increased in all of the species, with the rats showing the greatest response. A 4-week average of the metabolic rate for mice and hamsters ranged from 17 to 24% above the controls. The oxygen consumption increase in rats ranged from 32 to 84%. The increase in metabolic activity did not significantly affect the survival ratio or the incubation time of the disease.

The results of 16 experiments in which the animals had propylthiouracil in their diet are summarized in Table II. Metabolic rates were measured on experiments 1, 6, 9, 11, 12, 14, 17, and 18. It was difficult to demonstrate a uniform depression of oxygen consumption by propylthiouracil in mice. The mean 30-day metabolic rate for the PTU group ranged from an increase of 2.2% to a decrease of 4.0% from the normal control group. This could have been due to their restlessness in the metabolic chamber where increased activity may have masked the effect of the propylthiouracil. The metabolic rate was depressed from 0.8 to 29% in hamsters and guinea pigs, and 26% for rats after ingestion of this drug. The incubation time and the survival ratio varied from experiment to experiment between the animals receiving the drug and those that did not, but no consistent trend could be noted.

Table III summarizes five experiments in

which the animals were thyroidectomized, and one in which the pituitary gland of rats was removed. Sprague-Dawley rats weighing approximately 100 gm each were hypophysec-

tomized and inoculated 9 days later. The thyroidectomized animals were allowed at least 14 days to recuperate before being inoculated with rabies virus.

TABLE I. The Effect of Feeding Iodinated Casein^a on the Mortality and Incubation of Rabies.

Expt. no.	Animal species	Survival ratio ^b			Av no. of days incubation			Av metabolism ^c (ml of O ₂ /kg ^{3/4} per min)		
		N ^d	T	Diff.	N	T	Diff.	N	T	Diff. (%)
10	Mice	5/12	6/12	+1	10.6	11.8	+1.2	ND	ND	—
13	Mice	8/10	6/10	-2	15.5	15.0	-0.5	ND	ND	—
14	Mice	1/18	1/18	0	10.2	9.7	-0.5	37.7	46.8	+24
9	Mice	4/12	6/12	+2	11.4	12.0	+0.6	36.9	43.1	+17
11	Ham	2/12	2/12	0	15.8	15.3	-0.5	22.7	28.0	+23
12	Rats	9/12	10/12	+1	14.7	17.5	+2.8	24.3	32.0	+32
15	Rats	7/12	6/12	-1	11.4	12.5	+1.1	20.0	36.9	+84

^a 0.1% in the feed taken *ad libitum*.

^b Numerator denotes number of animals surviving; denominator denotes number of animals inoculated.

^c Average of tests taken on four animals (6 mice) two times each week over a period of 4 weeks.

^d Abbrev.: N means normal or untreated group; T means treated animals; Diff. means the difference of the treated group from the control group; and ND means not done.

TABLE II. The Effect of Feeding Propylthiouracil^a on the Mortality and Incubation of Rabies.

Expt. no.	Animal species	Survival ratio ^b			Av no. of days incubation			Av metabolism ^c (ml of O ₂ /kg ^{3/4} per min)		
		N ^d	T	Diff.	N	T	Diff.	N	T	Diff. (%)
4	Mice	6/18	12/18	+6	12.8	12.2	-0.6	ND	ND	—
5	Mice	0/20	4/20	+4	9.4	10.8	+1.4	ND	ND	—
17	Mice	0/12	2/12	+2	9.9	9.3	-0.6	38.7	38.8	+ 0.3
18	Mice	8/12	10/12	+2	14.3	21.0	+6.7	34.8	33.7	- 3.2
9	Mice	4/12	4/12	0	11.4	12.0	+0.6	36.9	37.7	+ 2.2
8	Mice	4/12	4/12	0	10.7	11.2	+0.5	ND	ND	—
10	Mice	5/12	8/12	+3	10.6	9.7	-0.9	ND	ND	—
13	Mice	8/10	8/10	0	15.5	19.0	+3.5	ND	ND	—
14	Mice	1/18	2/18	+1	10.2	10.3	+0.1	37.7	36.2	- 4.0
3	Ham	1/8	0/8	-1	15.3	10.7	-4.6	ND	ND	—
7	Ham	0/8	0/8	0	11.7	14.1	+2.4	26.3	20.2	-23
11	Ham	2/12	0/12	-2	15.8	10.2	-5.6	22.7	22.1	- 2.4
1	G. pig	3/14	4/14	+1	19.1	18.2	-0.9	10.6	7.5	-29
2	G. pig	3/10	1/10	-2	17.1	16.3	-0.8	ND	ND	—
6	G. pig	0/8	0/8	0	14.9	14.4	-0.5	13.4	13.3	- 0.8
12	Rats	9/12	9/12	0	14.7	10.7	-4.0	24.3	18.0	-26

^a 1.0% in feed taken *ad libitum*.

^b Numerator denotes number of animals surviving; denominator denotes number of animals inoculated.

^c Average of tests taken on four animals (6 mice) twice each week over a period of 4 weeks.

^d Abbrev.: N means normal or untreated group; T means treated animals; Diff. means the difference of the treated group from the control group; and ND means not done.

TABLE III. The Effect of Thyroidectomy and Hypophysectomy^a on the Mortality and Incubation Time of Rabies.

Expt. no.	Animal species	Treatment	Survival ratio ^b			Av no. of days incubation			Av metabolism ^c (ml of O ₂ /kg ^{3/4} per min)		
			N ^d	T	Diff.	N	T	Diff.	N	T	Diff. (%)
3	Ham	Thyroidectomy	1/8	1/8	0	15.3	16.4	+1.1	ND	ND	—
1	G. pig	Thyroidectomy	3/14	4/14	+1	19.2	15.2	-4.0	10.6	9.4	-11
2	G. pig	Thyroidectomy	3/10	3/10	0	17.1	16.9	-0.2	ND	ND	—
15	Rats	Thyroidectomy	7/12	6/12	-1	11.4	12.7	+1.3	20.0	17.6	-13
16	Rats	Hypophysectomy	4/12	3/11		15.4	12.1	--3.3	24.6	19.2	-21

^a Thyroidectomy performed 14 days before experimentation. Hypophysectomy performed 9 days before experimentation.

^b Numerator denotes number of animals surviving; denominator denotes number of animals inoculated.

^c Average of tests taken on four animals (6 mice) twice each week over a period of 4 weeks.

^d Abbrev.: N means normal or untreated group; T means treated animals; Diff. means the difference of the treated group from the control group; and ND means not done.

The mean metabolic rate was decreased 11 and 13% in thyroidectomized guinea pigs and rats. Hypophysectomized rats showed a 21% decrease in rate. Although the metabolic rate was uniformly decreased by these methods, the survival ratio was essentially the same in the control and experimental group.

Discussion. This was an investigation into the hypothesis that the metabolic rate of the host may have a direct influence on the incubation time of rabies virus in several species of animals. To study this relationship, artificially-induced metabolic changes were made in experimental animals by the use of drugs or surgery. By feeding iodinated casein, an increase in metabolic rate was demonstrated within 24 hours. Except for rats, propylthiouracil did not produce remarkable results in the species tested and required approximately 14 days to show its maximum effect. This may be due to the presence of stored thyroid hormone or to individual variations in drug response (7,8). The thyroidectomized rats had an average decrease of 13% in metabolic rate and the hypophysectomized rats had a 21% depression in their metabolic rate below the normal control.

While the animals receiving iodinated casein in their diet exhibited a marked increase in their metabolic rate, the number of animals surviving different doses of the rabies virus given by intramuscular inoculation did not

differ from those animals not receiving the drug. The mean of the incubation period revealed a small increase in the treated animals over the untreated controls, but this difference was greatly exceeded by the individual variation between animals in the same experiment whether they received the drug or not. In addition, these results did not seem related to the consistent increase in metabolic rate.

The apparently inconsistent results in 30-day average metabolic rates following the incorporation of PTU in the diet may reflect in part the way in which the data were obtained. That is, in experiments 6, 7, 9, and 11, the oxygen consumption rate was calculated much less frequently after day 15. Since the metabolic rate readings nearer the time of inoculation showed less difference than later ones, by averaging all the metabolic determinations the difference between groups was less apparent. Therefore, a straight line was fitted to metabolic rates for each PTU, control and CI group for the period from inoculation to 15 days postinoculation. Fifteen days was selected in order to determine whether a change was noticeable within a relatively short time after inoculation. Any change after 2 weeks would be of relatively little importance because of the short average incubation period for rabies in these animals. As a check on this procedure, experiments 1, 15, and 16, comparing metabolism of controls

TABLE IV. Regression Coefficients for Metabolic Rates, Day 0-15 after Rabies Inoculation, for Four Species of Animals, and Five Treatments.

Expt. ^c	Animal	Treatment ^d		
		PTU	Control	CI
17	Mice	-.85 ^b	-.12	—
18	Mice	-.67 ^a	-.23	—
9	Mice	-.95 ^b	-.44	.61
14	Mice	-.26	-.32	.57
2	G. pig	-.11	.03	
6	G. pig	-.04	-.13	
11	Ham	-.08	.10	.51
12	Rats	-.26	-.33	.60 ^b
		Thyroid	Control	
15	Rats	-.23	-.15	.62 ^{b,c}
1	G. pig	-.05	-.12	
		Hypoph.	Control	
16	Rats	.10	.18	

^a Significant deviations from $b=0$, at $p=.10$ level.

^b Significant deviations from $b=0$, at $p=.15$ level.

^c Started 10 days preinoculation.

^d PTU = Propylthiouracil (1% in diet); CI = Iodinated casein (.1% in diet); Thyroid = Thyroidectomized 14 days before experimentation; and Hypoph. = Hypophysectomized 9 days before experimentation.

* Expt. no. 7 excluded because only 3 readings were made.

with the metabolism of thyroidectomized or hypophysectomized animals were tested simultaneously. These experimental animal groups were assumed to have reached metabolic equilibrium by the time of the rabies inoculation, and in fact showed regression coefficients strikingly similar to those for controls (Table IV).

The analysis of variance for the slopes of the fitted regression lines does not show significant differences at the $p = .05$ level, but slopes less than zero (at $p \leq .15$) for PTU groups were obtained in Expts. 9, 17, and 18, and slopes greater than zero (at $p \leq .15$) for CI groups were obtained in Expts. 12 and 15. Although these differences are not statistically significant in the traditional sense, they are slightly suggestive of a difference.

The time from onset of symptoms to death in mice is significantly ($p \leq .05$) longer on the average in PTU groups than in CI or con-

trol groups. Whether this indicates an effect due to decreased metabolic rates, or to some other effect of PTU is not clear. It may suggest a sparing effect due to PTU, however, the influence of PTU may not be of sufficient magnitude (or speed) to prevent onset of symptoms.

Since the average time to onset can be excessively influenced by a single very early (or very late) observed day of onset of symptoms, additional tests were run on the data. A test of the ranks of the day on which each experimental and control group became 25% symptomatic indicates a definite ($p = .03$) but slight delay in the PTU-fed groups (in experiments on mice), but no difference between CI and controls. The suggestion of a delaying effect of PTU persists (at a lower level of significance, $p = .14$) in a test of the ranks of the day on which 50% of each group becomes symptomatic. The fact that CI and control groups do not differ statistically may reflect the small number of experiments and small number of animals in each experiment.

The guinea pig and hamster experiments showed no significant differences in time to onset of symptoms between any of the groups. It should also be noted that the challenge dose for hamsters and guinea pigs in some cases was apparently too large, and may have masked differences by a very rapid kill in all groups.

The metabolic rate after thyroidectomy in guinea pigs and rats, and after hypophysectomy in rats was appreciably lower than the rate in the controls. Rat Expt. 15 showed a significant ($p = .10$) delay to onset of symptoms in the thyroidectomized animals. However, rats hypophysectomized in Expt. 16 showed an earlier ($p = .08$) onset of symptoms. Whether this later fact contradicts the results of Expt. 15 or indicates additional "stress" (due to greater endocrine imbalance) in hypophysectomized rats is not clear.

In most cases the experimental groups did not differ significantly from controls. However, because relatively few experiments were run on hamsters, guinea pigs, and rats, no definite relationship was demonstrated between the incubation period and survival due

to a change in metabolic rates after rabies inoculation in these animals. The relatively larger experiments with mice, and the greater number of these experiments suggest a delaying effect to time of onset (due to PTU in the diet) and a consequent higher survival rate.

Summary. Studies were made to determine if raising or lowering the metabolic rate of experimental animals would increase or decrease the incubation period of rabies virus.

The metabolic rate of mice, rats, hamsters, and guinea pigs was increased from 17 to 84% with iodinated casein with no significant change in the incubation period or mortality rate of rabies when compared to control animals. The host metabolic rate was decreased by thyroidectomy, hypophysectomy, or propylthiouracil in the diet. In most cases the incubation period of the treated groups did not differ significantly from the rabies controls. However, in thyroidectomized rats with the metabolic rate decreased 13% there was a significant delay in the onset of rabies symptoms ($p = .10$). In mice fed PTU the mean metabolic rate ranged from a 2.2% increase to a 4% decrease. An analysis of 216 mice

suggests a delaying effect in the onset of rabies symptoms and a higher survival rate for mice with PTU in the diet than controls with a normal diet. It is suggested that the decreased metabolic rate of a host could delay virus propagation and give more time for active and passive treatment and possibly increase the chances of survival for an animal exposed to rabies virus.

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Yellow Fever Vaccine I. Development of a Vaccine Seed Free from Contaminating Avian Leukosis Viruses (32885)

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Chicken flocks are known to harbor avian leukosis viruses (ALV) which are transmitted from mother hen to egg. Special conditions of isolation and rearing are necessary to produce ALV-free eggs. Embryos of ALV-infected eggs and live virus vaccines prepared from these embryos are also contaminated. Rigorous control is exercised in the production of vaccines of chicken embryo origin (e.g., measles) and additional careful testing of the final vaccine insures a product which is ALV-free.

Yellow fever (YF) vaccine currently available in the United States is known to be con-

taminated with ALV. This vaccine is produced on a "seed lot system" (1), i.e., primary seed virus is used to prepare lots of secondary seed which, in turn, is utilized for the preparation of vaccine lots. The primary seed (lot AB237) which is ALV-contaminated was produced in an era (middle 1940's) when little was known of ALV contamination of eggs. In order to make ALV-free YF vaccine, it would be necessary to develop an ALV-free primary seed and to produce all subsequent secondary seeds and vaccines in ALV-free eggs.