TABLE I.
Distribution of Poliomyelitis Virus After Right Infraorbital Nerve Dip. Four Cynomolgus Monkeys. Cam Strain of Virus.

Days after inoculation	Nasopharyngeal washings	Stools	Semilunar ganglia		Washed pharyngeal wall
			Right	Left	
2	0	0	—	—	—
3	+*	0	—	—	—
4	+	+	+	0	0

* Non-paralytic case: cord histologically positive.
— Not tested.
\+ Clinically positive: cord sections positive.
0 Cord sections negative; symptoms absent or very questionable.

excreted and detected in the overlying mucus.

Our experiments tentatively confirm the hypothesis in the following respects: (1) ascent by centripetal spread to the Gasserian ganglion; (2) limitation of infection to the nerve system in question during the first 3 days; (3) excretion of virus in the appropriate area on the third day. Avoidance of primary exposure of the mucosa to virus rules out implantation of virus on the mucosal surface. Excretion by the lymphatics is highly improbable, since lymph flow is away from rather than toward the surface.[9]

The very early appearance of virus on the nasopharyngeal mucosae is noteworthy and interesting in reference to similar findings in the human disease,[1-3] as is the fact that it appeared a day later in the stools than in the nasopharyngeal washings, which might be explained on the basis of swallowing.

This work is to be repeated and expanded.

Summary. Poliomyelitis virus when applied to the central end of a divided branch of the trigeminal nerve in the cheek travels centripetally to the corresponding semilunar ganglion within three days. Centrifugal spread to the nasopharyngeal surfaces, which are supplied by the trigeminal nerve, was demonstrated by detection of virus in the nasopharyngeal washings on the third and fourth days. Virus was also found in the stools on the fourth but not on the third day, suggesting that it had been swallowed. It is suspected that excretion, like invasion, of poliomyelitis virus occurs through axonal channels.

9 Yoffey, J. M., and Drinker, C. K., *J. Exp. Med.*, 1938, **68**, 629.

15998

Prolonged Survival of Adrenalectomized-Nephrectomized Rats on a Low Potassium Diet.*

PHILIP K. BONDY AND FRANK L. ENGEL.†
From the Department of Medicine, Emory University School of Medicine, and the Medical Service, Grady Hospital, Atlanta, Ga.

Adrenalectomized animals are extraordinarily sensitive to all types of stress, including the metabolic derangements following nephrectomy. During the course of investigations on the relation of the adrenal cortex to urea synthesis, considerable difficulty was met in maintaining life in adrenalectomized-nephrectomized animals long enough for satisfactory study. The limited survival was not influenced by the use of liberal amounts

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† Present address: Department of Medicine, Duke University, Durham, N.C.

of desoxycorticosterone acetate and saline for as long as seven days preoperatively. Durlacher and Darrow[1] have already shown that the survival of nephrectomized but otherwise normal rats may be significantly prolonged by preoperative potassium depletion by a low potassium diet. Since the adrenalectomized rat is particularly sensitive to potassium accumulation, such as occurs after nephrectomy, the effects of a low potassium diet on the survival of adrenalectomized-nephrectomized rats were investigated. It was found that not only was the survival time prolonged over that of adrenalectomized-nephrectomized rats on the chow diet, but it was also significantly extended beyond that of nephrectomized, but otherwise normal rats also on a low potassium diet.

Methods. Male rats of the Sprague-Dawley strain, weighing 170-200 g, were fed Rockland rat chow until the beginning of the experiment. For 3 days before operation the animals received the following diet: Casein (Technical), 180; sucrose, 570; peanut oil, 220; cod liver oil, 10; brewers' yeast, 20; bone ash, 20; sodium chloride, 5. This diet contains 1.4 mEq of potassium per 100 g,‡ as compared to 24.6 mEq per 100 g of Rockland rat chow. The animals were allowed free choice of water and 1% saline in their drinking fountains throughout the experiment. On the evening of the third day, all food was removed, and the animals were anesthetized by the intraperitoneal injection of 3 mg nembutal per 100 g of body weight. After operation, the animals were bled 2 to 3 times daily from the tail, 0.3 ml of blood being removed and used for the determination of urea nitrogen by the xanthydrol method of Engel and Engel.[2] Total urea synthesis, expressed as milligrams of nitrogen per 100 g body weight per hour was calculated from the rise of blood urea nitrogen, assuming the urea to be equally distributed throughout the body water, *i.e.* 75% of the body weight. Survival times were judged to be the last time the rats were known to be alive. Thus rats dying during the night were considered to have a shorter survival time than was actually the case. This error equally affected both adrenalectomized and control animals. The largest error introduced in this assumption was not more than 6 hours. Urea levels at the time of death were calculated by assuming no change in the rate of urea formation from the time of the last determination to the time of death. In several instances where samples were obtained within an hour or less of the time of death, this assumption was found to be valid.

Nephrectomies and adrenalectomies were performed simultaneously through bilateral subcostal incisions. In several animals, the adrenal area was examined histologically after death and the absence of adrenal rests demonstrated.

Results. Table I compares the survival time of nephrectomized-adrenalectomized rats on the chow diet and previously treated with 5 mg of desoxycorticosterone acetate for 3 days with those on the low potassium diet, and with nephrectomized rats with intact adrenals on the low potassium diet. It is apparent that preparation with the special diet prolonged the life of the adrenalectomized-nephrectomized rats to a significant degree ($p<0.01$).§ There was also a significant increase in the survival time of adrenalectomized rats as compared to those which had merely been nephrectomized ($p<0.02$). When Upjohn's aqueous adrenal cortical extract‖ was administered to the adrenalectomized rats at a dose level of 1 ml twice daily through the course of the experiment, the survival time was intermediate between the control and the adrenalectomized group, but was not significantly different from either group ($p>0.05$).

1 Durlacher, S. H., and Darrow, D. C., *Am. J. Physiol.*, 1942, **136**, 577.

‡ The potassium analysis was performed by the Department of Biochemistry, Emory University.

2 Engel, M. G., and Engel, F. L., *J. Biol. Chem.*, 1947, **167**, 535.

§ Fischer's tables of p were used in determination of statistical significance. A value of p equal to or less than .02 is considered significant.

‖ We are indebted to Dr. D. J. Ingle of the Upjohn Co. for generous supplies of aqueous adrenal cortical extract.

TABLE I.
Survival Times and Terminal Blood Urea Nitrogen Levels After Nephrectomy.

	No. of rats	Survival time, hr	Terminal blood urea nitrogen, mg/ 100 ml
Adrenalectomized-nephrectomized, chow diet*	3	13.5 ± 2.3†	—
Adrenalectomized-nephrectomized, low potassium diet	7	98.4 ± 5.2	240 ± 5.1†
Nephrectomized, low potassium diet	7	77.1 ± 5.5	329 ± 35.92
Adrenalectomized-nephrectomized, low potassium diet, adrenal cortical extract	7	89.1 ± 3.4	264 ± 17.4

* Treated with 5 mg desoxycorticosterone acetate for 3 days prior to nephrectomy.
† Standard error of the mean.

The relation of the survival time to the degree of uremia is also shown in Table I which records the blood urea nitrogen concentration of the rats at the time of death. It was found that the final urea concentration of the adrenalectomized animals was lower than that of the non-adrenalectomized group, even though the survival time of the adrenalectomized group was longer. Once again, the adrenalectomized group treated with adrenal cortical extract fell into an intermediate position. The difference between the adrenalectomized and control animals was significant ($p<0.01$); those between the group given adrenal cortical extract and the other two were not ($p>0.10$).

When the rate of urea synthesis in the nephrectomized rats was compared with that of the nephrectomized-adrenalectomized rats, as shown in Fig. 1, no difference was found initially, but as the experiment progressed increasing and significant differences were found. There was a significant rise in the rate of urea formation in animals with intact adrenals, but the apparent fall in the adrenalectomized animals' rate was not significant.

Discussion. By depleting rats of potassium, it has been possible to maintain life long enough to study their rate of urea formation after adrenalectomy and nephrectomy. Two methods of potassium depletion are available; the use of the low-potassium diet, and the administration of 2 mg of desoxycorticosterone acetate per day for periods of 3 weeks.[1] We have elected to use the special diet because it is more convenient and less expensive than is desoxycorticosterone. It is probable that the high mortality noted in the group of rats on the chow diet was a result of an inadequate preoperative preparation with desoxycorticosterone, since at this dose level the period of preparation was too short to effect potassium depletion.

The 3-day preparatory period we have used probably does not achieve a maximal protective effect, since Durlacher and Darrow's rats[1] prepared with a similar diet for 21 days survived an average of 112 hours as compared to 77 hours for nephrectomized rats with intact adrenals in our series.

Selye and Nielsen[3] have reported increased survival time in rats pretreated with 10 mg of desoxycorticosterone acetate for only 3 days before nephrectomy. In their series, however, the increased survival time may have been due to the functional adrenalectomy produced by such large doses of desoxycorticosterone. The rate of urea formation of the pretreated animals was lower than that of the controls, suggesting a general depression of adrenal cortical function in this experiment.

It is of interest that, after potassium depletion, adrenalectomized rats survive nephrectomy for longer periods than do rats with intact adrenals. This is the only instance of which we are aware in which the adrenalectomized animal is more resistant to a stress, *i.e.* nephrectomy and fasting, than is the animal with intact adrenals. It should be emphasized, however, that the adrenalectomized potassium-depleted rat is not more resistant to uremia, since the blood urea nitrogen level at the time of death was lower

[3] Selye, H., and Nielsen, K., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 541.

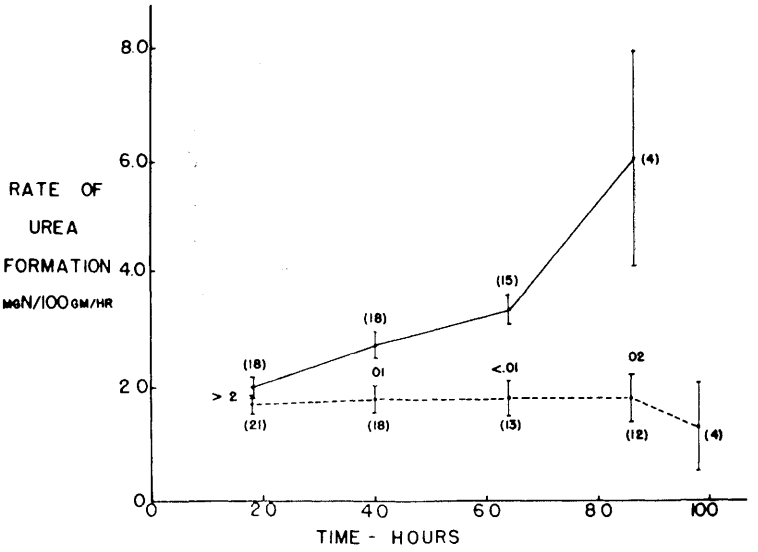

Fig. 1

The rate of urea nitrogen formation per 100 g body weight per hour after nephrectomy in the control (solid line) and adrenalectomized (dashed line) rats maintained on a low potassium diet for 3 days before nephrectomy. The numbers in parentheses refer to the number of observations at each point. The vertical bars represent the standard error of the mean of each point and the numbers between the curves the p values at each point.

in the adrenalectomized than in the control rats. The administration of adrenal cortical extract shortened the survival time of adrenalectomized rats. These effects can all be explained on the basis of the depression of protein turnover, and consequently, of urea formation observed after adrenalectomy. In the control group, the rising rate of urea production was probably due to the usual protein catabolic response to stress which occurs in the presence of the adrenal cortex. Harrison and Long[4] found that the rate of nonprotein nitrogen excretion increased after the second day of fasting in normal rats. The increasing difference in the rate of urea production between the adrenalectomized-nephrectomized and normal nephrectomized rats in our experiment is of the same degree of magnitude as found by Harrison and Long in their studies on nitrogen excretion. Thus starvation itself may be a sufficient explanation for the rising rate of urea production. The metabolic adjustments of uremia may play little or no part in accelerating urea formation in the nephrectomized rat with intact adrenals.

Summary. Adrenalectomized-nephrectomized rats fed a chow diet before operation have a high mortality rate. If the animals are prepared preoperatively for 3 days on a low-potassium diet, the adrenalectomized animals survive longer than rats with intact adrenals. The rate of production of urea is normal immediately after adrenalectomy, and fails to accelerate in the manner characteristic of rats which intact adrenals subjected to starvation and uremia. The blood urea nitrogen level at death is lower in adrenalectomized than in control animals. Administration of adrenal cortical extract to adrenalectomized-nephrectomized rats tends to restore the survival time and terminal blood urea nitrogen level to control values.

[4] Harrison, H. C., and Long, C. N. H., *Endocrinology*, 1940, **26**, 971.