On this diet the average survival period of adrenalectomized rats from our colony and of this age was 16 days. In order to shorten the survival period, a solution of 0.5% KCl was given in the drinking bottles in place of tap water. This reduced the average survival period to 8.1 days.

The results of this study are given in Table I. It is evident that the adrenalectomized rats receiving extracts of blood and urine from patients with Cushing's disease survive a longer period that the untreated controls. An attempt has been made to estimate roughly the potency of these extracts by comparison with known amounts of ACH (Upjohn). It would appear that 1 cc of blood from a patient with Cushing's disease contains an amount of ACH equivalent to slightly more than the hormone content of 4 g of fresh adrenal glands. A 24-hour urine specimen may contain an amount equivalent to 400 g of tissue. The urine of the patient with the adrenogenital syndrome showed no significant amount of ACH; however the patient did excrete androgenic hormone as follows per 24 hours: 5 IU of free androgenic hormone and 33 IU of hydrolyzable androgenic hormone.

The question might be raised whether the presence of oestrin or of prolan, which may both be present in the urine, might prolong the life of adrenalectomized rats, but Grollman and Firor found that these substances had no effect upon prolonging the lives of these animals. This is being checked further in a special study.

Conclusion. Patients with Cushing's disease have in their blood an excess amount of a substance which resembles adrenal cortical hormone in its ability to prolong the life of the adrenalectomized rat. Furthermore, this substance is excreted in significant amounts by the kidneys.

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Effect of Vitamin A Deficiency upon Rate of Pupil Dilation During Dark Adaptation.

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Wald has recently shown that there is a definite chemical relationship between visual purple in the retina and vitamin A. The

visual processes of light and dark adaptation form a cycle, vitamin A being the breakdown product of visual purple, as well as the precursor. It is found mainly in the light adapted retina, and only a trace exists in the dark adapted one. The latter, however, contains relatively large amounts of visual purple.[1, 2]

Wald has further shown that bleached isolated retinas contain much more vitamin A than retinas from light adapted animals. He concludes from this that the visual purple system loses vitamin A and relies upon the diet for its replacement. These results form an excellent basis for the explanation of night blindness caused by vitamin A deficiency.

If the stimulation of the rods, in which the visual purple is contained, has anything to do with the pupillary reflex, the measuring of the rate of dark adaptation, determined by changes in pupil diameter, might form the basis for an objective test for the determination of night blindness.

The experimental work done thus far consists of the measuring of the rate of pupil dilation during dark adaptation, by a method previously described.[3] This, in brief, involved the constricting of the pupil by an intense stimulus light followed by the taking of a series of photographs in virtually complete darkness, by means of an infrared source of light. The rate of pupillary constriction was also determined in the latter parts of the experiments, using a method described in a later paper.[4]

Nine rabbits, from 6 to 8 weeks of age, were placed on the vitamin A-free diet, consisting of rolled oats, 60 parts; dry skimmed milk, 30 parts; and dry brewer's yeast, 10 parts. A few drops of Viosterol were administered by mouth every week.

A series of the photographic measurements was taken to determine the pupil dilation during dark adaptation on each animal before it was placed on the restricted diet. The group was then divided into 2, and the measurements were repeated every week, taking each half alternately.

The diet and measurements were kept up for 92 days, during which time weight records of each animal were obtained. There was a steady rise in weight until the 56th day, when 2 of the rabbits showed a loss in weight. By the 70th day, all the animals showed

1 Wald, G., *J. Gen. Physiol.*, 1935, **18**, 905.

2 Wald, G., *J. Gen. Physiol.*, 1935, **19**, 351.

3 Gullberg, J. E., Olmsted, J. M. D., and Wagman, I. H., *Am. J. Physiol.*, 1938, **122**, 160.

4 Gullberg, J. E., Olmsted, J. M. D., and Wagman, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 616.

a marked decline. The animal which showed the greatest decline died on the 64th day, and exhibited clear evidence of vitamin A deficiency. On the 81st day, 2 more animals showed marked symptoms, including clouding of the cornea and swelling of the eyelids. The animal which showed the most severe signs was fed pure carotene every day for 5 days. Definite response to the administration of carotene was evident on the 2nd day, and there was a progressive improvement until, on the 6th day, all of the superficial symptoms of the deficiency had disappeared.

An analysis was made of all the data from the pre-deficient series to the clear-cut terminal stages with the physical lesions. No significant difference in the rate of response could be detected at any point in the series. All the curves of the rate of pupil change during light and dark adaptation showed the form characteristic of the normal animals previously described.[3]

Our results are in disagreement with those of Phillips and Bohstedt who produced a syndrome in rabbits which they attributed to a deficiency of vitamin A in the diet. Among other symptoms they found that "the light reflex was sluggish and somewhat disturbed."[5]

The evidence that has been collected does not exclude the possibility of an effect existing in vitamin A deficiency which could only be revealed by a change in pupillary response to low threshold stimuli. Further work along these lines has been planned.

Laurens concludes that the pupillary reflex is primarily and more easily elicited from the cones, but that stimulation of the rods also gives it.[6] It seems to us that, with the intense, broad beam of light used as the stimulus for pupillary constriction in our experiments, the cones alone must be responsible for the pupillary reflex. If the rods have anything to do with pupillary action, the effect is probably not detectable by the method herein described.

[5] Phillips, P. H., and Bohstedt, G., *J. Nutrition*, 1938, **15**, 309.

[6] Laurens, H., *Am. J. Physiol.*, 1923, **64**, 97.