cell bodies was observed only in the original cultures. Fiber regeneration occurred in four successive subcultures, made over a period of 25 days, but the extent of the outgrowth diminished with each passage. The decreased size of the explant with each transfer may be a partial cause for this latter effect, since a portion of the tissue is invariably lost at each transfer.

It may appear somewhat surprising that biotin in such dilute concentrations should have a marked effect on the proliferation of embryonic cells, in view of the large amounts of biotin known to be present in eggs.[4] Presumably, the chick embryos, from which the embryonic extract was prepared, should contain quantities of biotin, unless the vitamin is used up by the cells as fast as it is absorbed from the yolk. However, it is not unprecedented to find that very minute amounts of biotin can produce a considerable effect. For example, Du Vigneaud, *et al.*,[5] find that, in rats, the addition of 2-4 γ per day of biotin to an ordinarily protective diet was sufficient to increase greatly the incidence of tumors produced by the carcinogenic compound, "butter yellow."

In view of the fact that biotin is actively synthesized by many species of bacteria and molds and is apparently important in their nutrition,[6] it is hoped that the vitamin may prove valuable in producing higher titers of virus or larger yields of rickettsiae in culture. Experiments are in progress to test these possibilities.

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Protective Action of Desoxycorticosterone Acetate and Progesterone in Adrenalectomized Mice Exposed to Low Temperatures.

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The early work of Wyman and Tum Suden[1] with autoplastic trans-

4 Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

5 DuVigneaud, V., Spangler, J. M., Burk, D., Kensler, C. J., Sugiura, K., and Rhoads, C. P., *Science*, 1942, **95**, 174.

6 Landy, M., and Dicken, D. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 449.

* This work was started at the Roche-Organon Laboratories, Nutley, New Jersey.

1 Wyman, L. C., and Tum Suden, C., *Am. J. Physiol.*, 1929, **84**, 362.

plants of cortical tissue in adrenalectomized rats indicated a rôle of the adrenal cortex in the maintenance of body temperature. This work was followed by the use of cortin in preventing a drop in colonic temperature of adrenalectomized rats (Hartman, *et al.*[2]). Recently it was shown that corticotropin could protect hypophysectomized but not adrenalectomized rats against cold.[3]

Widström,[4] and Selye and Schenker[5] suggested the use of the cold exposure test as a quick and accurate method for the bioassay of cortin. The latter used adrenalectomized rats (35-50 g) and 12 to 24 hours after operation exposed the animals to a low temperature. Treatment was started at the time of exposure and was spaced at 2-hour intervals. By this technic they were able to detect amounts as small as 0.03 cc of cortin (Wilson preparation). In view of this action of cortin and the sensitivity of the test, it seemed warranted to study the influence of desoxycorticosterone acetate (D C A).† A modification in technic was employed. This modification consisted in treating the adrenalectomized mice previous to their exposure to cold. The D C A was dissolved in an oil vehicle instead of water.

Experimental. Young adult female mice were adrenalectomized and on the same day received the first of 4 injections of D C A or control substance. The following day they received the next 2 injections, and on the third day, the fourth injection was given. Three groups of mice received D C A in doses of 100 γ, 250 γ and 500 γ, each dose being dissolved in 0.1 cc of peanut oil. The total amount of hormone administered was 0.4, 1.0, and 2.0 mg respectively, per mouse. Of 4 other groups of adrenalectomized mice, one received no treatment, one received 500 γ of progesterone, and a third and fourth were given 0.1 and 0.2 cc of peanut oil per injection.

One hour after the last injection, the mice were put into small wire mesh cages and placed in either a refrigerator or cold room at $6 \pm 2°C$. The experiment was further controlled by subjecting both normal, untreated adrenalectomized, and sham operated mice to cold. The animals were observed hourly and the number and percentage of survivors noted.

Discussion. Preliminary experiments, using the Selye-Schenker

[2] Hartman, A. A., Brownell, K. A., and Crosby, A. A., *Am. J. Physiol.*, 1931, **98**, 674.

[3] Tyslowitz, R., and Astwood, E. B., *Am. Physiol. Proc.*, 1941, **133**, 427.

[4] Widström, G., *Acta Med. Scandinav.*, 1935, **87**, 1.

[5] Selye, H., and Schenker, V., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 518.

† The hormones used were prepared by Roche-Organon.

procedure which involves treatment during exposure to cold, gave negative results for desoxycorticosterone acetate dissolved in oil. For this reason the precold treatment schedule was used and its success can probably be explained on the basis of slower absorption of D C A from oil than cortin from a water solution. Furthermore, the new procedure was designed to give the mice time to recover from the shock of the operation.

It was observed that in the cold-exposure test adult animals could be used as readily as immature ones. Apparently removal of a major portion of the adrenal tissue is sufficient to insure death in 100% of the mice within 6 hours after exposure to low temperature; whereas, at room temperature a number were found to survive indefinitely. These showed adrenal rests at autopsy. The average life span of successfully adrenalectomized mice in the strain used in the present report is about 5 days, which agrees with the figure of 3 to 7 days given by Pfeiffer and Hooker.[6]

The mortality curves (Fig. 1) show little protection by D C A at the dose level of 100 γ per mouse, but at 250 γ and 500 γ excellent protection was observed. It is probable that a dose smaller than 250 γ but greater than 100 γ would also give significant protection. Pfeiffer and Hooker[6] showed that 250 γ of D C A could maintain life in adrenalectomized mice at room temperature. Since a daily dose of

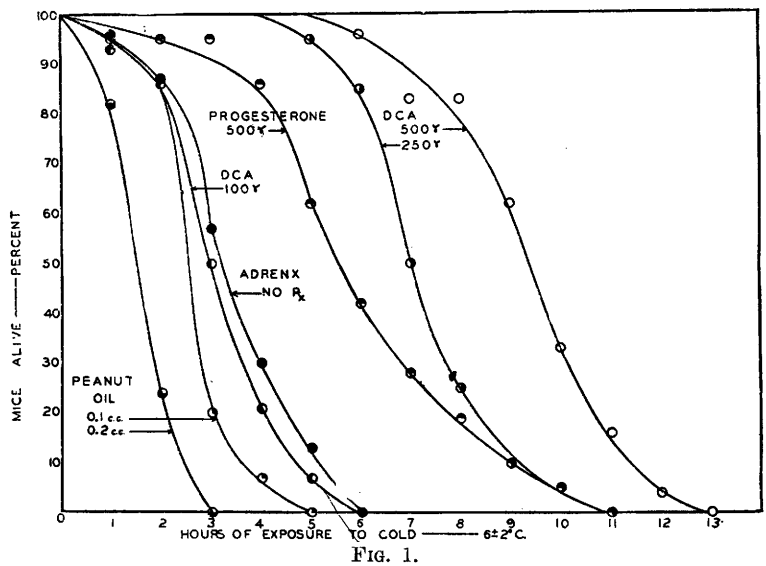

FIG. 1.

Mortality curves of adrenalectomized mice exposed to cold (6 ± 2°C). A total of 179 mice were used and each curve is based on 17 to 24 mice.

[6] Pfeiffer, C. A., and Hooker, C. W., *Am. J. Physiol.*, 1940, **131**, 441.

1 mg of D C A is required to maintain the adrenalectomized rat[7] and 0.5-1.0 mg to maintain the adrenalectomized guinea pig[8] at room temperature, and since the entire cold exposure test requires 3 days, this procedure offers certain advantages with respect to sensitivity and time.

It is a known fact that progesterone can prolong the life of adrenalectomized animals. In the present experiment, progesterone was tested at the dose level of 500 γ (Fig. 1) and a mortality curve obtained somewhat similar to the D C A curves. Comparison of the protective action of progesterone and D C A at the 50% survival level indicates a ratio of 2.6 to 1.

Kendall[9] stated that compounds with an oxygen atom on C_{11} and those found in his amorphous fraction can maintain life in adrenalectomized rats subjected to low temperatures. He found that corticosterone and compound E were about equally active, namely, at dose levels of 16 γ and 20 γ respectively. The present data indicate that D C A also has a protective action, but at a dose level of 250 γ. This is a much greater amount than that of the former compounds and while it may be explained on the basis of a difference in action, *i. e.*, corticosterone is more effective in glycogen metabolism and D C A, in electrolyte metabolism, in this case it would appear that the difference is quantitative and not qualitative.

Control experiments indicate that peanut oil has a toxic effect. Mice receiving 0.2 cc of peanut oil showed a significant decrease in resistance to cold as compared with the untreated adrenalectomized mice. Beznak and Korenyi[10] reported that arachis oil increased the mortality of a "working rat population," and Bruce and Tobin[11] demonstrated the presence of a fraction in sesame oil that was toxic to adrenalectomized rats.

Summary. By the use of a precold treatment injection schedule it has been demonstrated that desoxycorticosterone acetate at dose levels of 250 γ and 500 γ and progesterone at 500 γ prolongs the life of adrenalectomized mice subjected to low temperatures. Peanut oil at the dose level of 0.2 cc has been shown to possess a significant toxic action.

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7 Grollman, A., *J. Pharm. and Exp. Therap.*, 1939, **67**, 257.

8 Clark, W. G., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 253.

9 Kendall, E. E., *Proc. Staff Meet. Mayo Clin.*, 1940, **15**, 297; *J. A. M. A.*, 1941, **116**, 2394.

10 Beznak, M., and Korenyi, A., *Arch. int. Pharmacodyn.*, 1941, **45**, 321.

11 Bruce, R. A., and Tobin, C. E., *Endocrinol.*, 1940, **27**, 956.