1,940 mg. and 3,060 mg., respectively, representing 58% and 76% of the total doses injected.

The other 3 dogs received, in addition to 375 mg. per kg. of sodium barbital by vein, a total dose of 2 cc. novasurol intramuscularly and 150 mg. per kg. of ammonium chloride subcutaneously. These injections produced profound diuresis in 2 dogs. The third died an hour after the administration; no diuresis had occurred, and 0.15% of the total injected dose was detected in the 23 cc. of urine produced. One dog lived 18 hours, in which time 7% of the total amount of barbital sodium injected was excreted. The last excreted 26% in 40 hours. In these experiments, diuretic measures contributed neither to the recovery of the animals nor to the excretion rate of barbital.

The study of the excretion of other malonyl ureas in the urine by this test and the application of the same in a clinical diagnosis of acute and chronic poisonings and in legal medicine will be reserved for the detailed communication.

The authors wish to express their thanks to Dr. John L. Gipprich, S. J., Regent of the Medical School, for his interest and criticism.

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Preparation of a Purified and Highly Potent Extract of Growth Hormone of Anterior Pituitary Lobe.

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Since the demonstration by Long and Evans[1] of the growth hormone of the anterior pituitary, various workers have confirmed the existence in alkaline extracts of the anterior lobes of animals of a growth-promoting substance. Van Dyke and Wallen-Lawrence[2] have suggested the use of totally hypophysectomized animals as a test object for determining the potency of extracts. The latter also made definite progress in the preparation of potent extracts which were less crude than those previously used.

We have been successful in preparing highly potent extracts of the anterior lobe of oxen. These extracts have been tested for

[1] Long and Evans, *Anat. Rec.*, 1921, **21**, 62.

[2] Van Dyke and Wallen-Lawrence, *J. Pharmacol.*, 1930, **40**, 413.

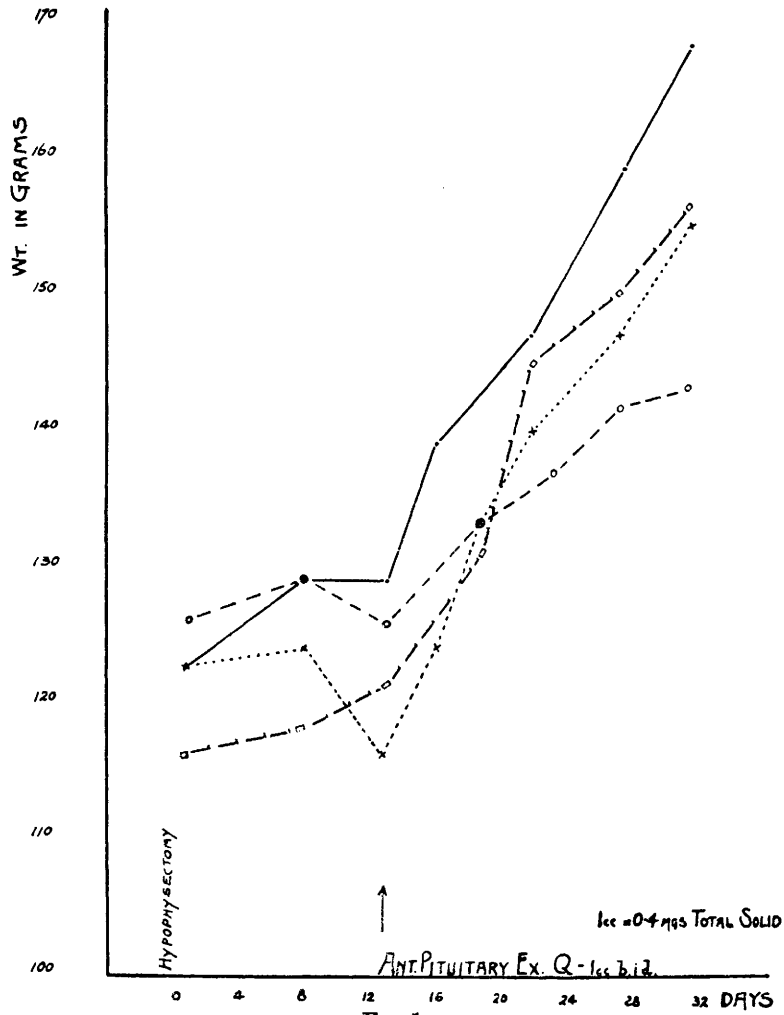

FIG. 1.

growth-promoting effects upon totally hypophysectomized rats. Growth has started almost immediately following the institution of therapy and has continued at a fairly uniform rate (see chart).

It was first observed that an alkaline extract (anterior lobes extracted with 10 volumes of 1% NH_4OH) which had been partially deproteinized, by the addition of acetic acid to pH 6.5 and filtering, had a slight growth-promoting effect in hypophysectomized rats. Such extracts were then saturated with ammonium sulphate in the presence of 1% NH_4OH, and the resultant precipitate collected, extracted with dilute ammonia and dialyzed. After dialysis the

solution was concentrated at low temperature and pressure. During the concentration process a semi-crystalline substance separated out at a pH of 7.5 to 8. This was removed and an alkaline extract of it was found to be highly potent in stimulating growth in hypophysectomized rats.

From these leads the following method has been evolved. The anterior lobe tissue is treated with several volumes of dilute alkali: 0.5 to 1% NaOH or 1% NH_4OH. The mixture is acidified with acetic acid and filtered and the residue is again suspended in dilute alkali and reprecipitated with acetic acid and filtered. This extraction with dilute acetic acid as outlined may be repeated 5 times. Ammonia is added to the combined filtrates to give approximately a 1% concentration. By the addition of appropriate amounts of calcium chloride and sodium phosphate a suspension of $Ca_3(PO_4)_2$ is produced in the solution and the whole is concentrated at low temperature and pressure until the ammonia is practically all removed. The $Ca_3(PO_4)_2$ is then collected on a Buchner filter and is extracted repeatedly with 0.5% NaOH. The NaOH solution of adsorbed material which is thus removed is acidified with acetic to pH 6.5. It is then made alkaline with ammonia and concentrated at low temperature and pressure to remove the ammonia slowly. The semi-crystalline material which separates out at a pH 7.5 to 8 is removed and extracted with dilute sodium hydroxide. The alkaline solution is then almost neutralized and it may be used for assay at this point, 1 cc. representing approximately 2 gm. of original gland tissue. The total organic solids are between 1 and 2 mg. per gram of original anterior lobe tissue and ¼ cc. of such a solution administered twice daily to completely hypophysectomized rats has resulted in marked growth.