ascribed to treatment. It will be noted that the proportion of animals showing gross tumor lesions ranges from 29 to 75%, excluding the case of mice which had in addition an intravenous injection of a Seitz filtrate of a 0.9% NaCl extract of homologous tumor tissue. Likewise as to generalized reactions the frequency was of the same order of magnitude as was found with spontaneous tumors at the same cortisone dose level, except where supplementary treatment was given.

When terramycin, 0.1% in the drinking water was given the incidence of generalized reactions fell to zero. Penicillin in the dose employed did not show such an effect. Two points are of interest in this connection. First, it is apparent that terramycin did not reduce the frequency of tumor necrosis following cortisone. Second, it is apparent that terramycin reduced the frequency of generalized reactions and allows long survival.

The fact that survival can be expected following tumor necrotizing doses of cortisone when accompanied by terramycin suggests that by combining with such treatment another agent differentially more damaging to tumor than to host tissues, more complete tumor destruction might be accomplished. Studies using radiation, chemical agents and antigenic materials to such an end are already in progress.

Conclusions. In mice bearing spontaneous and transplanted mammary carcinoma it is possible by treatment with cortisone to induce a high frequency of massive necrosis with or without hemorrhage in the tumors. Cortisone administration in necrotizing doses is also followed by inflammatory lesions in lungs, liver and kidneys, which are usually fatal. Terramycin treatment before and during cortisone administration resulted in an abolition of the above-mentioned generalized reactions. It has not been possible with cortisone alone or with terramycin to obtain complete tumor destruction. It is pointed out that cortisone necrotizing treatment in conjunction with some other agent having differential damaging effects upon tumor and host respectively, may yield better results.

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Differentiation of Growth Hormone from the Pituitary Factor Which Produces Diabetes. (19531)

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The diabetogenic effect of pituitary extracts, observed by Houssay(1), was attributed by Evans to the growth hormone(2). It is now generally accepted, despite earlier evidence to the contrary(3), that growth hormone is the factor in pituitary extracts which is responsible for the production of diabetes(4-7). Extensive experimentation has yielded clear evidence that the pituitary contains a potent diabetogenic agent, or combination of agents, which rapidly produces severe insulin-resistant diabetes when administered to susceptible animals, and which produces permanent diabetes with pancreatic damage after prolonged administration(2,8). The diabetogenic effect may be obtained in the

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adrenalectomized dog(9) and is, in the intact animal, a far more potent effect than is induced by corticotropin, which may cause transient hyperglycemia. The acceptance of growth hormone as the pituitary diabetogenic factor stems largely from the belief that the purified growth hormone preparations which produce the effect, including those which are crystalline and electrophoretically homogenous(10,11), are indeed chemically and biologically pure. Some further evidence, based on the concomitant destruction of growth-promoting and diabetogenic activity, has suggested that the production of diabetes is an inherent property of the growth hormone(12).

It has now been found that the growth-promoting and diabetogenic activities of pituitary extracts may be differentiated, and that growth hormone, prepared as recently described(13), is largely freed of the diabetogenic factor.

Experimental. Preparation of growth hormone: As in the method of Astwood, Raben, and Payne for the purification of corticotropin (14), acetone-dried pig anterior pituitary powder† was extracted at 70°C with glacial acetic acid, thyrotropin, luteotropin, and gonadotropins were removed by precipitation with a half-volume of acetone in the presence of salt, and "crude corticotropin" was precipitated with one volume of ether(15). Two per cent of the solids containing 90% of the corticotropin was removed by stirring a 2.5% solution of the ether precipitate in 0.1 N acetic acid with an 8% weight of oxycellulose (12% carboxyl) for 24 hours(16). Retreatment with oxycellulose using a 20% weight of the adsorbent removed another 1% of the solids and much of the residual corticotropin. The unadsorbed fraction, largely freed of other pituitary hormones, contained the growth hormone, which was concentrated by a modification of Wilhelmi's procedure(11). To the solution of unadsorbed material was added, with vigorous continuous stirring, strong potassium hydroxide calculated to make the solution 0.3 N with respect to potassium. Glacial acetic acid was then immediately added until a permanent cloud formed. The pH at this point was about 10. Additional glacial acetic acid was slowly added until the pH was brought down to 8.5. Stirring was continued for one-half hour and the precipitate then removed by centrifugation. The procedure to this point was carried out at room temperature but the subsequent steps were performed in the cold. To the supernatant of the pH 8.5 precipitate, while being stirred in an ice-water bath, was added an equal volume of 95% ethyl alcohol. The addition of alcohol was made over a period of 15 to 30 minutes, and stirring was continued for an additional 30 to 60 minutes. The preparation was then allowed to stand overnight in a cold room at 4-5°C, and the precipitate was then collected on a sintered glass filter, washed with 95% alcohol and with acetone, and dried in a vacuum. This precipitate contained the growth hormone and represented about 12 to 15% of the total material in "crude corticotropin." The preparation was readily dissolved by acidification with hydrochloric acid to about pH 3.5; no loss of potency was noted when a solution was kept at this pH. The growth hormone preparation thus obtained was found by assay in the hypophysectomized rat to be about equal in growth-promoting activity to the preparations of Li and of Wilhelmi, and to the Armour growth hormone preparation (Lot 285-110)‡ with which it was compared for diabetogenic activity.

Estimation of diabetogenic activity. When 3 adult dogs injected with this growth hormone preparation in doses up to 10 mg/kg/day failed to develop glycosuria, the preparation was compared for diabetogenic activity with the Armour growth hormone preparation. A male dog weighing 8 kg (Fig. 1) after receiving single daily injections of 50 mg of the present growth hormone preparation for 6 days, and 100 mg for 2 days without glycosuria, was given the Armour preparation in a dose of 50 mg/day. Glycosuria appeared after the third injection and continued until the injections were stopped. Retreatment with our preparation in doses of 100 mg/day for

† We are grateful to Dr. David Klein of the Wilson Laboratories for supplies of pituitary powder.

‡ Kindly provided by Dr. E. E. Hays of the Armour Co.

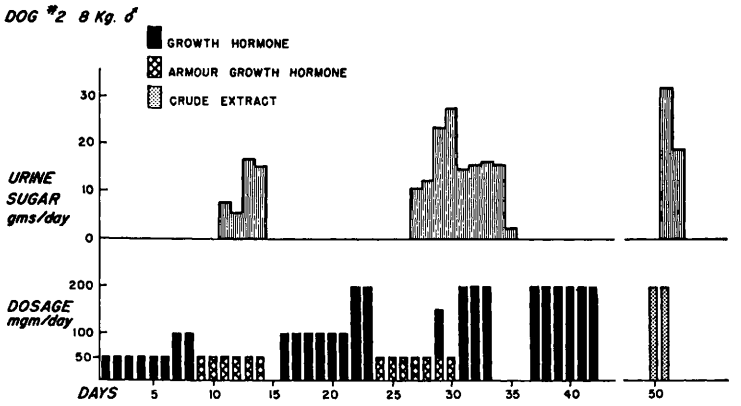

FIG. 1. Comparison of growth hormone prepared as described in the text, an Armour growth hormone preparation, and a crude extract in the production of glycosuria.

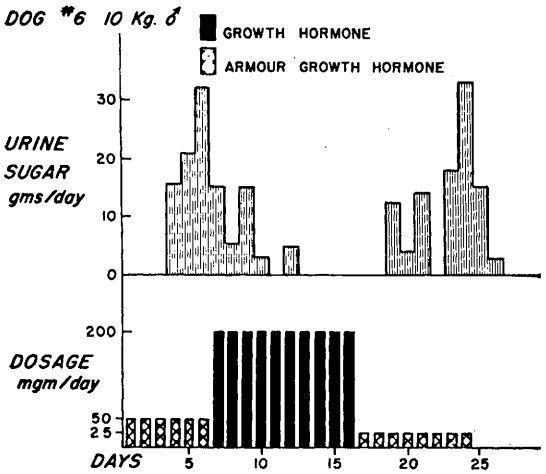

FIG. 2. Comparison of the growth hormone preparation and an Armour growth hormone preparation in the production of glycosuria.

6 days, and 200 mg/day for 2 days, again produced no glycosuria. A second course of the Armour material in a dose of 50 mg/day produced glycosuria beginning with the fourth injection. Glycosuria did not reappear during a third period of treatment with 200 mg/day of the present preparation, but did occur with the subsequent administration of the crude glacial acetic acid extract.

In another experiment carried out on a male dog weighing 10 kg (Fig. 2), the glycosuria which developed with the fourth injection of 50 mg of Armour growth hormone disappeared during 10 days of administration of the present preparation in a dose of 200 mg/day and recurred with Armour growth hormone in a dose of 25 mg/day.

Discussion. The difficulties involved in determining purity of pituitary hormones have been emphasized by recent experience with the purification of corticotropin(14). Isolation of the hormone was thought to have been achieved with preparations which were only 1% as potent as those subsequently produced. It now appears that diabetogenic material is present as a contaminant in growth hormone preparations which are crystalline and which behave as homogeneous substances when studied by solubility, diffusion, ultracentrifugation, and electrophoresis(10,11,17). The growth hormone prepared by the present method is probably not entirely freed of the diabetogenic principle, for large doses, while not producing glycosuria, did seem to delay the disappearance of established glycosuria (Fig. 1 and 2). The separation of the growth hormone from the factor which produces diabetes is nearly complete, however, and indicates the separate identity of these substances. The differentiation of the two activities serves to resolve the inconsistency in the concept that the metabolic processes which lead to growth also lead to diabetes.

Summary. Growth hormone was prepared from a glacial acetic acid extract of anterior pituitary powder. The product was equal in growth-promoting activity to other purified preparations but did not produce diabetes when administered in relatively large doses to

dogs, indicating that the diabetogenic factor of the pituitary is distinct from the growth hormone.

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Glucose Absorption in Highly Inbred Strains of Mice.* (19532)

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Studies on glucose absorption in the rat, beginning with the work of Cori(1), have raised a number of questions chief among which is the relation of absorption rate to the concentration of the administered solution. Cori(1) and others have claimed that absorption is essentially independent of concentration, while MacKay and Bergman(2), Fenton (3), and others found absorption to increase with increasing concentration of the administered solution. Two different colonies of rats were used by Fenton(3) with differing results. While both colonies showed dependence of absorption rate on concentration, this was much less pronounced in one of the colonies studied. It seemed that this colony difference might account for the divergent results which have appeared in the literature and might best be investigated in mice of highly inbred strains and of known genetic background.

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The demonstration that glucose absorption is accomplished by phosphorylation has led to speculation on the role of the widely distributed alkaline phosphatase in the absorption process(4). It was decided to investigate changes during glucose absorption in the concentration of phosphatase (using chemical methods) and in distribution (using cytochemical technics).

Methods. Female mice of the C57 and A strains, approximately 6-8 months old were fasted for 24 hours. Both strains were derived from the colony of Dr. L. C. Strong, Yale University, and were maintained by brother-sister matings. Following the fasting period 0.25 ml of glucose solution (25, 50 or 75% concentration) was administered by stomach tube. Exactly one hour later the animals were sacrificed and the contents of the alimentary tract (stomach, small and large intestines) washed into a volumetric flask. The glucose content of these washings was determined by the method of Somogyi(5). A group of 23 mice was fasted and then sacrificed without