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Hormonal Interactions Among GH, ACTH, Cortisol and Dexamethasone upon Size of Kidney, Liver, and Adrenal (38181)

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Our interest in the effect of hormones on weights of some of the major organs in the body was aroused by successful studies in hypophysectomized pigeons (1) (2) but similar studies in rats met with failure at those times. Studies with rats bearing a transplantable mammotropic pituitary tumor (MtT-F4) of Furth renewed our interest because such rats have livers and kidneys enlarged threefold relative to body weight. The rats with MtT have blood levels of GH and prolactin about 100 times and of ACTH about 1000 times that in normal rats. It was shown (3) that adrenalectomy prevents the enlargement of the kidneys and liver in rats with MtT-F4 thus proving the importance of hormones from the adrenal gland as the probable stimulants. The daily injection of large doses of the exogenous hormones GH, prolactin and ACTH alone and in combination confirmed that ACTH was the pituitary hormone most stimulatory of organ weight increase but that GH plus prolactin increased or augmented the effect of ACTH (4).

Experiments designed to elucidate the hormonal interactions of the diabetogenic hormones, bovine growth hormone (BGH), ACTH, cortisol and dexamethasone on the induction of glucosuria in rats with intact pancreases (5) resulted also in alteration in organ weights by day 5, when the rats were killed. In some experiments rats were killed daily over a 5 day period (6). This paper presents some of the more interesting organ weight data which are confirmatory of the effect of ACTH alone but also demonstrate the effect of cortisol and dexamethasone and the hormonal interaction among these hormones and with growth hormone. The studies were not designed to show effects on weights of adrenal, kidney, liver, thymus, pancreas and fat stores but did show such effects in the same hormonal dosage range that was effective in inducing glucosuria.

Materials and Methods. Bovine growth hormone (BGH), approximately 1 IU/mg, was obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases. Acthar gel (Armour) and ZnACTH (Organon) were used for ACTH. Tolbutamide was obtained from Upjohn Co. Dexamethasone (dexa) (1,4-pregnadien-9α-fluoro-16α-methyl-11β,17α,21 triol-3,20 dione) was a gift from Merck, Sharp and Dohme. Cortisol was purchased from Mann Laboratories. It was injected as an aqueous suspension of powder ($<$40 mesh) in 0.1 *N* sodium bicarbonate containing a drop of Tween-80. Injections were always given subcutaneously, usually twice daily for 5 days, and usually in a total volume of 0.5 ml/day of each hormone. When time studies were made with killings on days 1–5, injections were made every 8 hr.

Female rats of the Fischer strain were used. They were 3–5 mo of age and weighed 160–200 g before injection. Rats were treated in groups of 4 and fed Purina Rat Chow that had been ground to a coarse powder. Powdered tolbutamide was mixed with this diet at a concentration of 0.5%. The rats were placed in metabolism cages for collection of data on diabetogenesis (5). In the 5 day studies rats were killed in the

morning following the last injection, i.e., about 16–18 hr after the last injection. When time studies were done the rats were killed about 11 A.M., 2 hr after the morning injection. Organs were removed and weighed on appropriate balances.

A minimum effective dose (MED) to produce glucosuria by the fifth day was 200 μg/day for dexa and 10 mg/day of cortisol. Neither ACTH or BGH when injected alone could produce glucosuria in these rats having intact pancreases.

Serum prolactin and GH levels were measured using microtiter plates in a solid phase radioimmunoassay method (7). RIA reagents were obtained from the NIAMDD rat pituitary program.

Results. Effect of single hormones and combinations on adrenal weight. Adrenal glands from groups of untreated Fischer rats weighed in the range of 24–30 mg/100 g of body weight with standard errors of ± 1 or 2.

Adrenal glands from rats treated 5 days with large doses of cortisol or dexa regularly weighed in the range of 19–22 mg/100 g body weight with standard errors of ± 1 or less (Fig. 1). Most of the decrease in adrenal weight had occurred by the first day.

Adrenal glands from rats treated 5 days with 5 mg/day of BGH weighed 32–33 mg $\pm$ 1 on days 3, 4 and 5. This was a minimal increase and was not progressive with time (Fig. 1).

When treated with 80 IU/day of ZnACTH the adrenal weights increased daily to a mean weight of 137 $\pm$ 20 at 5 days. When 2 mg of BGH was injected with the ZnACTH, at most times, there was a significant increase in the adrenal weights above that found with ZnACTH alone, suggesting a synergistic interaction of the 2 hormones.

0.5% tolbutamide in the diet did not alter adrenal weights in 5 days, e.g., 25 $\pm$ 2 mg and 29 $\pm$ 1 mg. Nor did tolbutamide appear to affect the adrenal weights when they were stimulated by ACTH. Hence the adrenal weight data both with and without tolbutamide in the diet are plotted together in Fig. 2. A striking difference was found between Acthar gel and ZnACTH in ability to increase adrenal weights. A difference of nearly 10-fold was found dosewise.

Though BGH, dexa and cortisol had little effect or a negative effect on adrenal weight, when injected individually, all increased the adrenal weight increasing ability of Acthar, when injected with Acthar (Fig. 2). When both BGH and dexa were injected

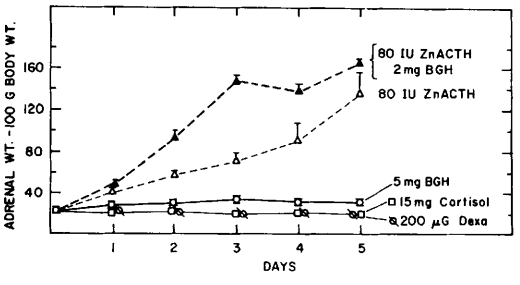

FIG. 1. Enhancement by BGH of the effectiveness of ACTH on increase of adrenal weight. Rats were injected every 8 hr. The total daily dosage is indicated. All vertical lines represent ± 1 SE.

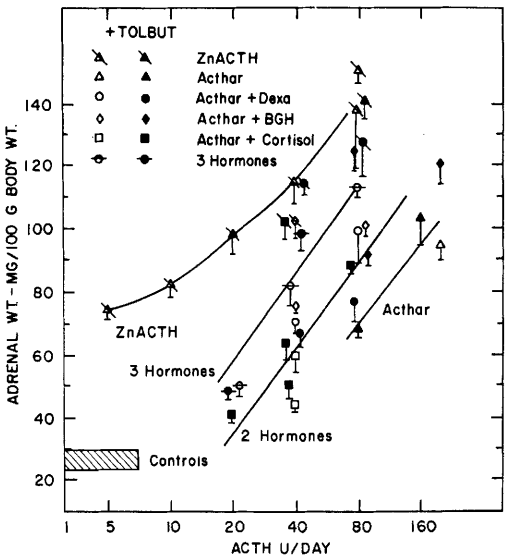

FIG. 2. Dose-response data of adrenal weight stimulation after 5 days injection with ZnACTH, Acthar or Acthar plus BGH, dexa or cortisol. The 3 hormone combination was Acthar + BGH (1 or 2 mg) + dexa (25 or 50 μg). Symbols that are filled represent data from groups of rats that had 0.5% tolbutamide in their diet. Vertical lines represent ± 1 SE.

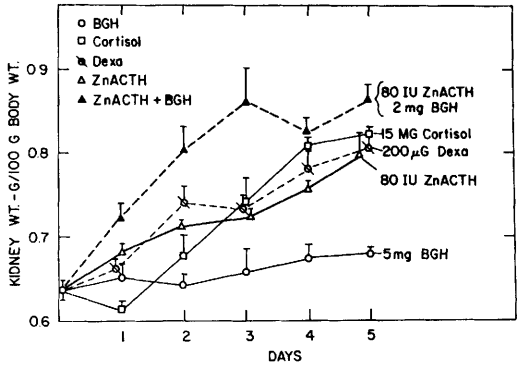

FIG. 3. Shows the effectiveness of dexa, cortisol and ACTH in stimulation of kidney weight and an enhancement of the effect of ACTH by BGH. Total daily dose is shown. Vertical lines represent ±1 SE.

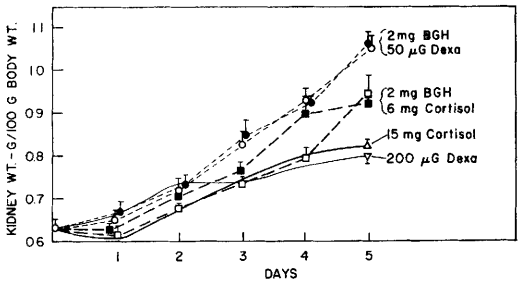

FIG. 4. Shows the enhancement of the kidney weight increasing effect of dexa and of cortisol by BGH. Total daily dosage is shown. The dexa and cortisol lines are from Fig. 4. The points with filled symbols were from rats on diet containing 0.5% tolbutamide. Vertical lines represent ±SE.

with Acthar there was a still greater increase in adrenal weight which was equivalent to about 3-fold, dosewise.

Effect of hormones on kidney weight. Kidney weight is directly controlled by products (glucocorticoids) of the adrenal (3). BGH at the 5 mg/day level increased only minimally the weight of the kidney (Fig. 3). Cortisol, dexa and ZnACTH in the large doses used all significantly increased kidney weight by day 5. The injection of 2 mg of BGH concomitantly with 80 IU of ZnACTH increased the kidney weight over that obtained by 80 IU of ZnACTH alone (Fig. 3).

Combination of BGH with either dexa or cortisol also produced a large augmentation in kidney weight (synergism) (Fig. 4). Note that the dosage of dexa was reduced by a factor of 4 and that of cortisol by 2.5 in the combination. When one group of 4 rats was injected 14 days with BGH (2 mg) and cortisol (6 mg) daily, kidney weights were 1.20 ± 0.04 g/100 g body weight and glucosuria was 4 g/day on day 14. The presence of 0.5% tolbutamide in the diet didn't appear to modify kidney weights.

Is there any correlation between kidney weight and the diabetogenic action of the hormones? In Fig. 3 there was some glucosuria on days 4 and 5 with cortisol and with dexa and the kidney weights were >0.75 g/100 g. When these hormones were combined with BGH (Fig. 4) there was a minimal glucosuria on day 2, when the kidney weights were between 0.7 and 0.75 g/100 g. This suggests a good correlation between kidney weight and glucosuria which is usually accompanied by a blood sugar >200 mg/100 ml. However (Fig. 3) ZnACTH and ZnACTH + BGH produced large kidneys, up to 0.85 g/100 g, without any glucosuria occurring, nor was there any elevation of blood sugar. In other words, these data (Fig. 3) show that a kidney size of 0.85 g/100 g doesn't necessarily result in glucosuria. BGH (5 mg/day) did not have any appreciable effect on blood sugar (6).

In Figs. 5–7 the kidney weights on day 5 of many treated groups of rats are plotted against the log of the dosage as dose-response curves. The presence of glucosuria on the fifth day is indicated. In none of the figures was glucosuria found until the kidney weight was >0.75 g/100 g. Arbitrary lines have been drawn to help the eye follow the points.

In Fig. 5 there is some indication that tolbutamide increased the effect of dexa on kidney weight. BGH, 1 mg, definitely synergized with the dexa and the effect of 2 mg was still greater. In the case of these mixtures tolbutamide made little difference. The MED values listed on Fig. 5 are the dosage of dexa alone or of dexa mixed with other materials, to induce minimal glucosuria by day 5 (5). These MED values are almost identical with the intercept of the lines drawn in Fig. 5 with a kidney weight value of 0.8 g/100 g. Dosewise there is an 8-fold

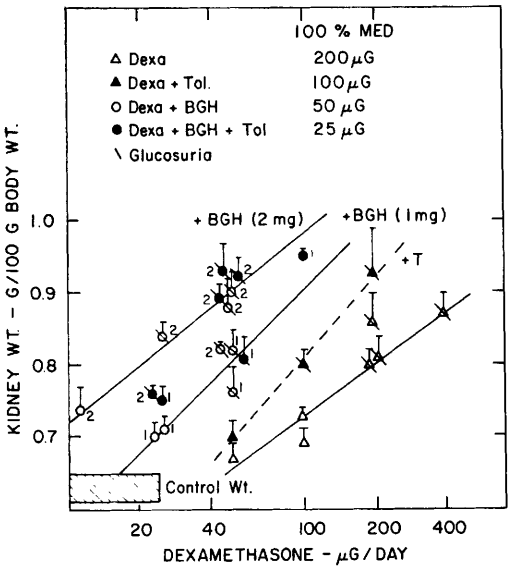

FIG. 5. Dose-response data of kidney weight vs daily dosage for 5 days of dexa and dexa plus (BGH) (1 or 2 mg/day). Filled symbols are from rats on 0.5% tolbutamide diet. The slash line indicates that rats had glucosuria on day 5. 100% MED indicates the minimum effective dosage of the various combinations to produce glucosuria on day 5 (5). Lines are drawn to aid the eye in locating points of similar combinations. Vertical lines represent +SE.

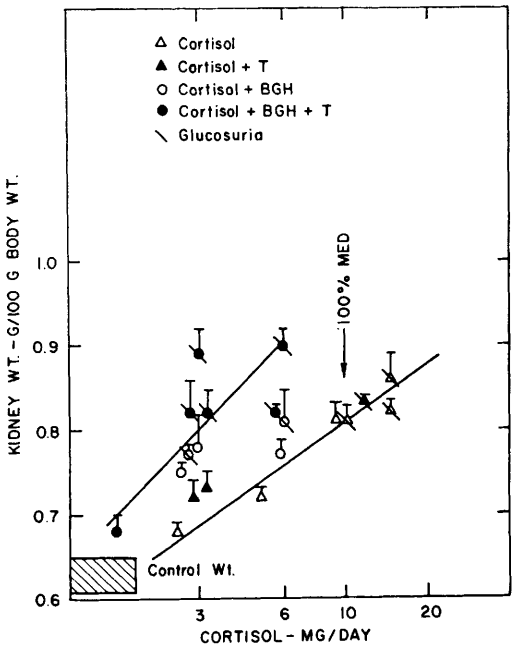

FIG. 6. Dose-response data of kidney weight vs daily dosage for 5 days of cortisol and cortisol plus BGH (1 or 2 mg/day). See legend to Fig. 6.

synergism on the basis of the dose of dexa when 2 mg of BGH was combined.

In Fig. 6 BGH is seen to augment the action of cortisol on kidney weight but only to the extent of 2 to 3-fold.

The data plotted in Fig. 7 show that Acthar and ZnACTH (except at lower dosages) are about equally effective in increasing kidney weight unlike their different effectiveness on adrenal weight. There is some synergism with BGH but only of the order of 2-fold. The interaction between Acthar and dexa or cortisol on kidney weight was found to be additive.

Dichotomy of action of ZnACTH and Acthar gel on adrenal and kidney weights. A straight line relationship between adrenal weight and kidney weight was found in rats bearing the transplantable pituitary tumor MtT-F4 of Furth (3). Hence all of our data was plotted with adrenal weight vs kidney weight (Fig. 8). It was found that a line with a slope of 1.8 passed near most

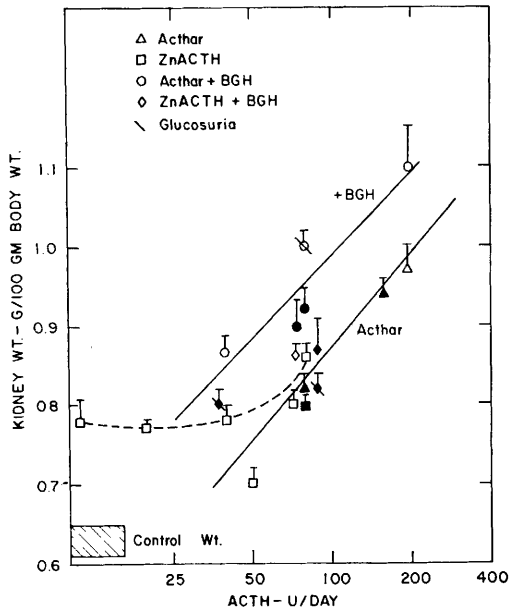

FIG. 7. Dose-response data of kidney weight vs daily dosage for 5 days of ACTH and ACTH + BGH (2 or 4 mg/day). See legend to Fig. 6.

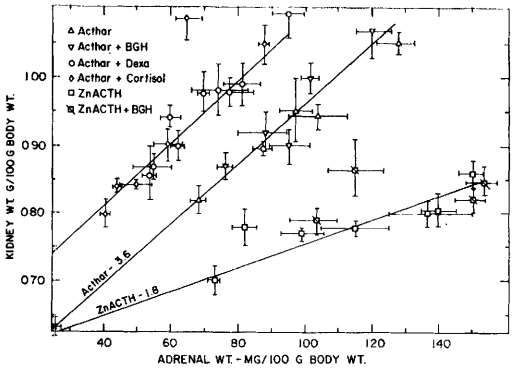

FIG. 8. Shows the relative increase of kidney and adrenal weights with various mixtures of hormones and especially the dichotomy in effectiveness of ZnACTH and Acthar gel on adrenal weight. 1.8 and 3.6 are the slopes of the lines. Vertical and horizontal lines represent ±1 SE.

of the points in which ZnACTH was used. A line with twice this slope (3.6) passed near most of the points in which Acthar was used. A line which passed through most of the points in which dexa or cortisol was combined with Acthar was found to have a slope of about 3.6 and to be parallel to the Acthar line but was displaced upward on the kidney axis by about 0.12 g/100 g body weight due to the inherent ability of dexa and cortisol to increase kidney weight but not adrenal weight. Thus this increased effect of the glucocorticoid combinations on kidney weight appeared to be the same at all Acthar dosages as they are reflected here by adrenal weight. Studies to show the combined action of ZnACTH and cortisol or dexa have not been done.

Effect of hormones on liver weight. That the presence of adrenal hormones are necessary for enlargement of the liver was also shown in adrenalectomized MtT-F4 tumors (3). Here BGH alone (5 mg/day) had little effect on liver weight (Fig. 9). ZnACTH, dexa and cortisol each increased liver weight at the dosage used. There was synergistic augmentation of liver weight when ZnACTH and BGH were both injected. In most cases the increase in liver weight occurred rapidly by days 1 and 2 with a slower progressive increase to 5 days. The largest weight increases in 5 days here were about 50%. This is not maximal since

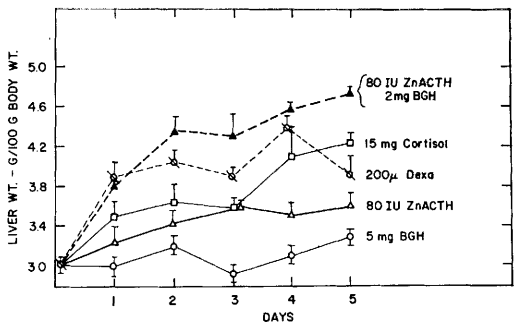

FIG. 9. Effectiveness of various hormones on increasing liver weight and showing enhancement of the effect of ACTH by BGH. Daily dosage is shown. Vertical lines represent ±1 SE.

MtT-F4 rats which are exposed to high endogenous hormonal levels of ACTH, BGH and prolactin for 3–6 wk have livers 3–4 times normal weight.

A dose-response plot of the data obtained with dexa and cortisol is shown in Fig. 10. On the basis of the MED to induce glucosuria dexa appears to be about twice as effective as cortisol dosewise in increasing liver weight, i.e., dexa is 100 times more potent than cortisol in ability to increase liver weight. By day 5, both Acthar gel (100 U/day) and ZnACTH (80 U/day) produced livers weighing about 3.6–4 g/

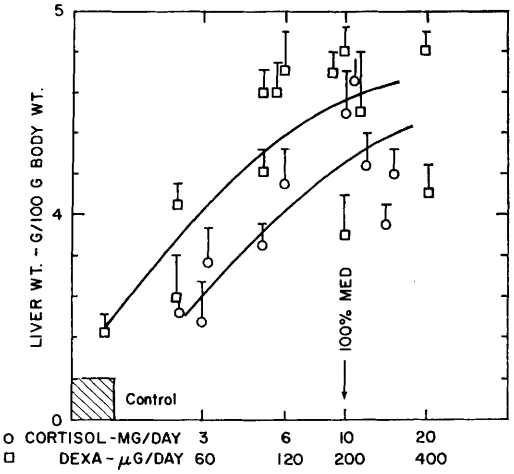

FIG. 10. Dose-response data of liver weight vs daily dosage for 5 days of dexa and cortisol. Minimum effective dose (MED) to induce glucosuria is 200 μg for dexa and 10 mg for cortisol. Vertical lines represent ±1 SE.

100 g without any glucosuria. They were about equally effective.

Other organs. The thymolytic action of ACTH and adrenal steroids is well known and is used as a bioassay method for glucocorticoids (8). At the large dose levels of ACTH and glucocorticoids used here the thymi weighed in the range of only 15–20 mg/100 g body weight on day 5. Weights of pancreas and the fat pad around the left horn of the uterus were also measured. The fat pad weights had a large coefficient of variation and showed only a slight tendency to decrease by 5 days. Pancreas weights were also variable. Since pancreas weight is largely the acinar tissue, weight changes are more related to food intake than to hormone dosage. The pancreas weight tended to decrease the first 2 or 3 days and then return toward the original weight as did the food intake (9).

The prolactin and GH levels were measured in the serums of rats in the experiments where rats were killed daily. No significant changes in prolactin level (45 ± 10 ng/ml) were found with BGH, cortisol, dexa, ZnACTH or tolbutamide. Growth hormone levels (60 ± 8 ng/ml) in the serum were not changed with dexa or cortisol. But ZnACTH (80 IU) and tolbutamide (0.5% and 2% in the diet, but not 0.1%) caused about a doubling of the GH level. All rats injected with BGH gave high estimates of GH (2–4-fold control levels). This was due to cross reaction of BGH with the rat GH reagents. It took 1,000 ng of BGH to equal the effect of 10 ng rGH but the lines were not parallel and no simple calculations were possible. The data suggested that serum levels of (BGH?) remained higher when BGH was injected with dexa than with cortisol but this needs to be repeated.

Discussion. The data in this paper confirm our earlier, one dose level data on the effect of injections for 10 days of BGH, ACTH and prolactin upon organ weights in normal rats (4) and our data in rats with MtT-F4 tumors (3). Here several dosage levels were used for only 5 days and glucocorticoids as well as ACTH and BGH were used.

The discrepancy between the dosages of the Zn and gel formulations of ACTH required to increase adrenal weight was unexpected because no such difference was found with kidney weight (Figs. 7 and 8) nor with induction of glucosuria (5, 6). The potencies of both of these commercial preparations had been determined according to the subcutaneous adrenal ascorbic acid depletion procedure described in USP XVIII, page 148, in which the ZnACTH preparation is acidified before injection. This acidification removes any benefit of the Zn formulation. Presumably both preparations contain purified powders of ACTH of similar purity and potency. The only difference then is one of formulation. One may deduce that the ZnACTH produces a more sustained blood level of ACTH than does the gel preparation.

The increase in kidney and liver weights and any glucosuria are reactions to products of the adrenal and secondary to the effect of ACTH on the adrenal gland. The dosage discrepancy of the 2 ACTHs in the primary affect (adrenal weight increase) and the lack of difference on the secondary effects requires an explanation. A qualitative or quantitative difference in the steroids produced by the adrenals are two possibilities.

Milkovic and Bates (10) found that the endogenous BGH and prolactin greatly modified the adrenal response to the high endogenous levels of ACTH found in MtT-F4. Blood levels of corticosterone were decreased and adrenal ascorbic acid levels increased by high levels of GH and prolactin in rats with large, ACTH stimulated adrenals. Milkovic *et al.* (4) confirmed these findings using exogenous hormones. Francois *et al.* (11) on the basis of *in vitro* incubations of adrenals from MtT-F4 rats implicated 18-OH-corticosterone or 18-OH-DOC as possible stimulants of organ weights but neither have been available in sufficient quantity to test *in vivo*. Bates and Garrison (12) found that when rats with MtT-F4 tumors were treated with amino-glutethimide and metyrapone in doses that reduced blood levels of corticosterone to normal that splanchnomegaly was still present sug-

gesting that the causal stimulant was an unusual adrenal hormone. Rats with MtT have kidney weights of 2 g/100 g body weight and liver weights of 10–12 g/100 g body weight. Rats with such large organ weights have been exposed to the endogenous hormonal stimuli for at least a month. In our experiments here the rats were treated with hormones for only 5 days and reached kidney weights of only a little over 1 g/100 g, and liver weights of less than 5 g/100 g body weight. In the one group treated 14 days with BGH (2 mg/day) and cortisol (6 mg/day) kidneys of 1.2 g/100 g and livers of only 4.6 g/100 g were obtained. The evidence points to an unidentified hormone from the adrenal as the growth stimulant of these organs.

There may be some significance to the fact that the large hormonal dosages used to induce glucosuria were in the same dosage range that increased organ weights. However rats with MtT-F4 tumors have larger organ weights and rarely have glucosuria unless partially pancreatectomized (13).

On the basis of many years of first hand experience we are convinced that kidney and liver size are controlled by hormones from the adrenal as is the adrenal weight by ACTH from the pituitary. Other hormones such as GH and possibly prolactin (not separately demonstrated) modify these hormonal actions perhaps by modifying pathways of steroid synthesis in the adrenal. The above facts have not yet been recognized by most books on the kidney and liver.

Summary. A ZnACTH preparation of ACTH was found to be nearly 10 times more effective than the Acthar gel preparation as a stimulator of increase of adrenal weight but the two preparations were equally effective as stimulators of weight increase of the kidney and liver. Bovine GH (5 mg/day) had little effect on adrenal, kidney or liver weight. When injected with either ACTH preparation the BGH further increased the adrenal weights. Either cortisol or dexamethasone also increased the effectiveness of Acthar on adrenal weight, and when BGH was injected as a third hormone a further adrenal weight increase was observed suggesting three different mechanisms of action. Kidney weights were increased by cortisol (15 mg/day), dexa (200 μg/day) or Acthar (100 IU/day) to about the same extent. The addition of BGH (1 or 2 mg/day) reduced by more than one half the dose of the adrenal hormone required to produce a given kidney weight. Liver weights were increased by ACTH, cortisol and dexa. BGH increased the effect of each of these hormones on liver weight. When ACTH was injected a linear correlation between adrenal weight and kidney weight was again demonstrated but different slopes were obtained with ZnACTH and Acthar. These data further indicate the importance of hormones and the complexity of their interaction (hormonal balance) in controlling size of various major organs and tissues in the body.

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