# Effects of Growth Hormone Administration on Hepatic Excretion of Bromsulfalein (BSP)* (33749)

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The skeletal and metabolic features of acromegaly, believed to be the end result of prolonged increased growth hormone secretion, are well known. A recent study from this laboratory documented a markedly increased transport maximum (T*m*) for bromsulfalein (BSP) in 11 acromegalic subjects (1). This uniform finding was the first indication of enhanced hepatic function in acromegaly. Ikkos *et al.* (2) Gershberg *et al.* (3) and others had previously shown increased kidney function as measured by renal plasma flow, glomerular filtration rate, and proximal tubular functions. The present authors undertook chronic administration of growth hormone in a series of dogs in an attempt to define the effects of growth hormone on hepatic handling of BSP. The results of this study are the basis for the present report.

*Procedure.* Six pure-bred, 8-month-old beagle dogs were used. Previous experience with this breed indicated individually consistent and reproducible hepatic function with respect to BSP kinetics (4). The treated group consisted of three dogs; matched littermates served as controls. Data for only two control animals are included in the present report because the third control was "lost to follow-up" early in the course of the study.

At the beginning of the study and regularly thereafter the following measurements were made: body weight, plasma glucose, intravenous glucose tolerance, and serum phosphate. Routine liver function tests were done periodically as were liver biopsies and paw X-rays. Hepatic function was assessed quantitatively in these unanesthetized dogs by measurement of BSP transport maximum (*Tm*) and relative storage capacity (*S*) with the dual infusion technique of Wheeler and his associates (5); the precise method was described previously (4). In general, these measurements were made at monthly intervals.

Calculation of BSP *Tm* and *S* was based on solution of the following simultaneous equations for the two infusion periods (I, II):

$$I_{I,II} = Tm \pm \Delta P_{I,II}(PV + S),$$

where $I_{I,II}$ are the BSP infusion rates (mg/min), $P_{I,II}$ the change in plasma BSP concentration (mg/100 ml per min), and *PV*, the plasma volume in hundreds of milliliters.

Growth hormone was obtained through the courtesy of the National Institutes of Health. For the first 6 months of treatment the hormone administered was bovine in origin. Subsequently, ovine growth hormone was used. After reconstitution of the crystalline, powdered hormone in distilled water to a concentration of 1/mg/ml and a pH of 9.0 by addition of 0.1 *M* NaOH, the solution was divided into 15-ml aliquots which were kept frozen until the day of administration. The initial dose was 0.25 mg/kg of body weight subcutaneously 3 times/week. After the first 6 weeks of treatment the dosage was increased to 0.5 mg/kg of initial body weight 5 times/week.

*Results.* All animals under observation gained weight during the period of study. However, weight gain was greater in all the treated dogs as noted in Fig. 1. The average increase for the first 9 months of treatment was 11.7 lb as compared to 4 lb in the controls. It is of interest to note that the two treated male dogs gained 13 and 16 lb respec-

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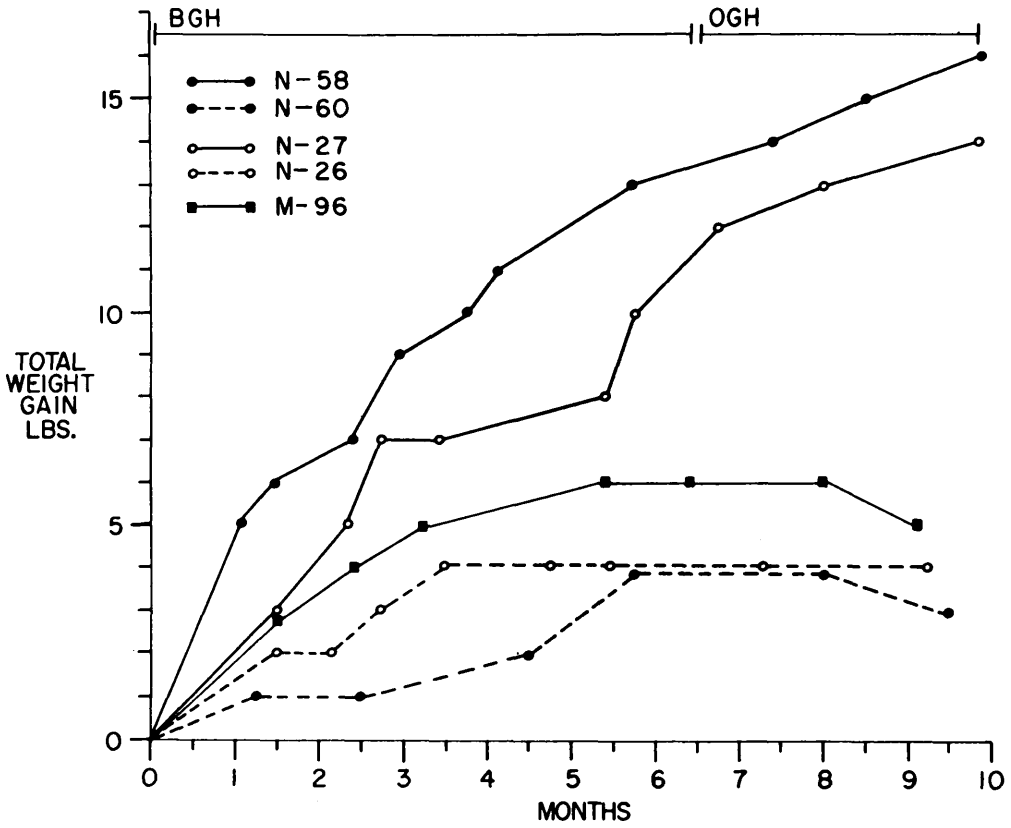


FIG. 1. Weight gain observed in the treated animals (solid lines and closed symbols); same data for their controls (broken lines and similar open symbols); bovine growth hormone (BGH); ovine growth hormone (OGH).

tively, and that the treated female gained only 5 lb.

Serum phosphate and plasma glucose levels did not differ significantly between the control and treated groups (Table I). Intravenous glucose tolerance was measured after 3 and 6 months of treatment and was normal in all animals. The lack of a diabetic response in young dogs receiving growth hormone was noted previously (6). X-Rays of the paws showed epiphyseal closure and bony configuration to be similar in both groups of animals. After 6 months of treatment the epiphyses were closed in all animals. No significant difference in foot pad thickness was noted between the control and treated groups.

Serum glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase were normal on all occasions. Liver biopsies obtained with a Menghini needle also were normal by light microscopy. However, such specimens were much too small to attempt to evaluate changes in cell size or number.

Measurement of the hepatic storage capacity ($S$) for BSP in the treated animals revealed no pattern of change distinct from that seen in the control animals. The results listed in Table II indicate a rather wide variation in $S$ and in only one instance (N-27) was there a remarkable increase in $S$ during the period of growth hormone administration. By contrast all members of the treated group displayed a consistent and marked increased in BSP transport maximum ($Tm$) during the initial 3 months of treatment. These results are tabulated in Table II and plotted in Fig. 2 as the percentage of the initial $Tm$. In the treated dogs excretory capacity for BSP increased by 58% on the

TABLE I. Fasting Blood Sugar and Serum Phosphorus Levels in Growth Hormone Treated and Control Dogs.

| | Treated | | | | | | Controls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N-27 | | N-58 | | M-96 | | N-26 | | N-60 | | |
| Month[a] | FBS | $PO_4$ | FBS | $PO_4$ | FBS | $PO_4$ | FBS | $PO_4$ | FBS | $PO_4$ | Remarks |
| 1 | | | 77 | 6.0 | 109 | 4.9 | | | 104 | 5.3 | BGH 0.25 mg/kg TIW |
| 2 | 113 | 5.3 | 87 | 5.5 | 90 | 4.3 | 94 | 4.8 | 92 | 5.6 | BGH 0.5 mg/kg OD |
| 3 | 89 | 6.9 | 83 | 4.8 | 91 | 6.5 | 90 | 4.3 | 92 | 5.6 | N-26, N-27: GTT, normal; N-58, N-60: liver biopsy, nl. All dogs: paw X-rays, nl. |
| 4 | 99 | 5.1 | 75 | | | | | | 86 | 4.7 | |
| 5 | 100 | 4.3 | 91 | 3.9 | 88 | 3.0 | 92 | 4.9 | 97 | 4.5 | N-58, N-60: GTT, normal; all dogs: paw X-rays and liver biopsies, normal |
| 6 | | | 100 | 3.5 | 106 | 3.2 | | | | | OGH begun |
| 7 | 112 | | 84 | 3.4 | | | 88 | 3.5 | | | |
| 8 | 99 | 4.2 | | | 90 | 3.1 | | | 106 | 4.5 | |
| 9 | 98 | 4.1 | 90 | 3.1 | | | | | | | |

[a] Month = month of treatment in progress; FBS = fasting blood sugar (mg/100 ml); $PO_4$ = serum phosphorous (mg/100 ml); GTT = glucose tolerance test; TIW = three times weekly; and OD = five times weekly.

average (range 38–76%) in the first 3 months of the study. In the ensuing 3 months the *Tm* fell in all these animals to an average level just 16% (range 8–23%) above the initial determination. No such variation in *Tm* was observed in either of the control animals whose BSP transport maxima were slightly increased (N-60, + 10%) and decreased (N-26, −15%), respectively. These results strongly suggested that the prolonged administration of growth hormone initially induced an increase in hepatic excretory function similar

TABLE II. Transport Maximum (*Tm*, in mg/min) and Hepatic Storage Capacity (*S*, in mg/mg %) in Growth Hormone Treated and Control Dogs.

| | Treated | | | | | | Controls | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N-27 | | N-58 | | M-96 | | N-26 | | N-60 | |
| Month | *Tm* | *S* | *Tm* | *S* | *Tm* | *S* | *Tm* | *S* | *Tm* | *S* |
| 1 | | | | | | | | | | |
| 2 | | | 2.6 | 18 | 2.9 | 36 | | | 3.1 | 25 |
| | | | | | | | | | 3.4 | 25 |
| 3 | 3.1 | 18 | 2.9 | 23 | 3.5 | 21 | 4.8 | 24 | | |
| | 4.3 | 13 | 3.6 | 15 | 3.9 | 35 | | | 3.3 | 26 |
| | 5.0 | 36 | | | | | | | | |
| 4 | 4.4 | 20 | 3.3 | 24 | 5.1 | 14 | 4.1 | 14 | | |
| 5 | 3.0 | 23 | 3.7 | 25 | | | | | 3.5 | 15 |
| 6 | 3.6 | 28 | 3.2 | 17 | 4.0 | 16 | 4.8 | 16 | 3.4 | 14 |
| 7 | 5.2 | 24 | 2.2 | 15 | 3.1 | 19 | | | | |
| 8 | 4.9 | 24 | 3.4 | 27 | | | 3.8 | 25 | 3.8 | 16 |
| 9 | 5.6 | 30 | | | | | | | | |
| 10 | 6.5 | 43 | | | | | | | | |

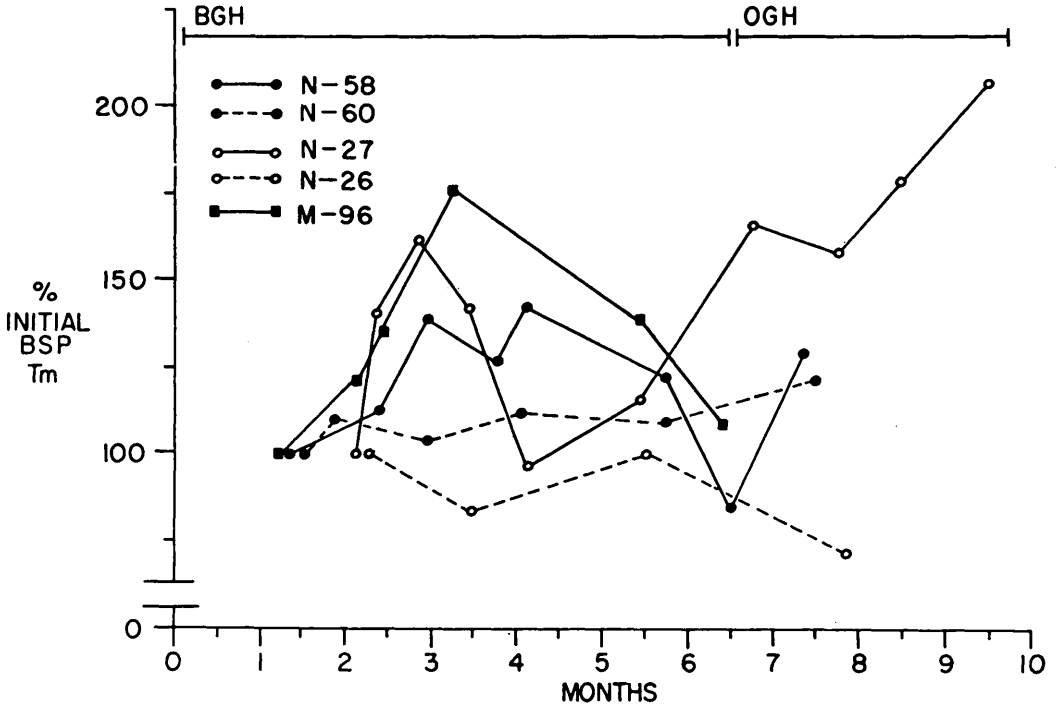


FIG. 2. Changes in BSP transport maximum (*Tm*), plotted as the percentage of the initial measurement, are shown for treated and control dogs (see Fig. 1 for explanation of symbols).

to that seen in acromegalic man.

From the middle of the sixth month of treatment, ovine growth hormone at the same dosage level was substituted for bovine material in all treated animals. During the next 3 months of treatment one treated animal (N-27) displayed a secondary and greater increment in *Tm* to nearly twice its initial value; again *S* displayed no similar increase. One other treated animal (N-58) showed an initial similar change, but technical difficulties in repeated venous catheterization prevented closer observation of both the remaining treated and control animals.

The possibility that the observed increment in *Tm* was a direct and immediate effect of growth hormone was evaluated in the following experiments. Constant infusions of BSP were administered to two unanesthetized dogs each of which was equipped previously with a Thomas duodenal cannula to permit direct collection of bile. During the course of the study plasma samples were drawn every 5 min and the biliary output of BSP was measured every 10 min. After a suitable control period, 30 mg of bovine growth hormone were administered intravenously during a 10-min period. The results of one such study appear in Fig. 3. There was no significant change in *Tm* BSP during the growth hormone infusion or in the following 30-min period. Consequently a direct immediate effect on the liver was not demonstrated. The relatively constant slope of the plasma concentration of BSP indicated further that increased extrahepatic loss of BSP was also unlikely as a direct acute effect of growth hormone.

*Discussion.* The origin of the observed changes in hepatic excretory function is unknown as are the underlying causes for those observed in acromegalic man. However, the increase in BSP *Tm* observed in these experimental animals is similar to that found in acromegaly and lends support to the idea that growth hormone may play a role in producing this change.

Previous measurements of the biliary *Tm*

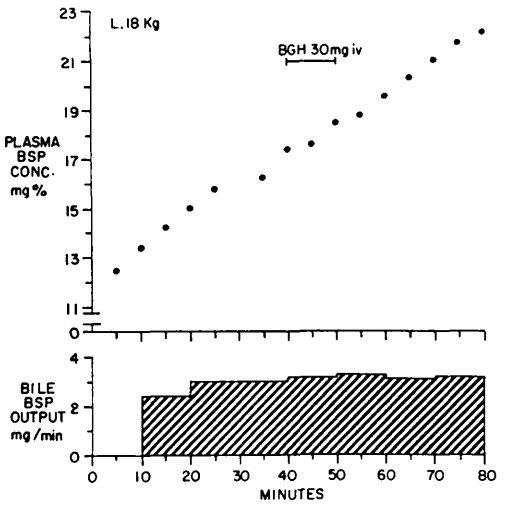


FIG. 3. Plasma concentrations (●), and biliary excretion (hatched area) of BSP, measured during a constant infusion of BSP, are shown before and after intravenous administration of 30 mg of bovine growth hormone (BGH).

for BSP by the method used in this study indicated a direct relationship between body weight and BSP *Tm* in the dog (5). It is, however, apparent that the observed changes in *Tm* were not related simply to the effect of increasing body weight, since the dog (M-96) with the smallest weight gain during the first 3 months of treatment showed the greatest increase in excretory capacity. It is possible that this increased biliary excretion of BSP was due to hepatic enzyme induction similar to that observed with other classes of compounds (7). In keeping with the latter hypothesis are the facts that in the liver BSP is largely conjugated with glutathione, a reaction catalyzed by an enzyme, and that conjugation may, in some species, facilitate biliary transfer of the dye (8).

Conversely, these changes in BSP transport may reflect anatomical changes induced by growth hormone. Although it is not certain whether the enlarged acromegalic liver is characterized by more or larger hepatocytes, or both, each possibility could account for the more effective transport of BSP in the growth hormone treated animal. Recent tissue culture studies have demonstrated hyperplasia induced by growth hormone in some species. However, in other cell lines only increased cellular metabolism was found (9). Consequently the cellular effects of growth hormone are probably multiple with the observed functional result reflecting a combination of these influences.

The pattern of BSP excretion observed after the initial increment is of further interest. All treated animals showed a decrease toward the initial *Tm* value. The appearance of antibodies to bovine growth hormone could have been responsible for this decrease in excretory capacity. Chronologically this would correspond well with the appearance of human growth hormone antibodies in human subjects receiving human growth hormone (10, 11). Furthermore, the increment again observed in BSP *Tm* when ovine growth hormone was substituted for the original bovine material, suggested that the latter was no longer effective although the liver was still responsive to stimulation by growth hormone of another species.

The significance of the effect of growth hormone on BSP kinetics remains to be elucidated. However, recent work by Gartner and Arias (12) showed that the hypophysectomized rat has a significantly decreased excretory capacity for conjugated bilirubin and displays cytochemical changes in bile canaliculi. Both of these defects were corrected by the administration of growth hormone. In addition, Beher and his associates (13) reported impaired excretion of bile acids in similarly prepared animals. Thus, growth hormone may have a regulatory role in hepatic function under normal circumstances. Whether enhanced hepatic function relative to endogenous materials follows growth hormone administration as in the case of BSP remains to be demonstrated. Such a hypothesis was postulated to explain the lack of clinical features of Cushing's syndrome in patients with acromegaly in whom elevated cortisol production rate was reported (14).

*Summary.* Biliary transport maximum (*Tm*) and relative storage capacity (*S*) for BSP have been measured in three dogs receiving parenteral bovine growth hormone. During the initial 3 months of administration a

58% increase in *Tm* was observed without a similar change in *S.* Thereafter, a decrease in *Tm* to nearly original levels was observed. Administration of growth hormone from another species (ovine) again produced a rise in *Tm* in at least one of the same animals. Evidence suggests that growth hormone exerts a regulatory influence on hepatic excretory function.

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# Mitomalcin: A New Proteinaceous Antileukemic Antibiotic Produced by *Streptomyces malayensis* (nov. sp.)* (33750)

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(Introduced by J. J. Oleson)

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We wish to report the isolation of a streptomycete from Malayan soil capable of producing a fermentation metabolite which exhibits highly significant activity against murine lymphocytic Leukemia L-1210 (1). This culture, classified as a morphologically unique streptomycete, is given the name *Streptomyces malayensis.*

A highly stable variant,[1] isolated from a glucose (1%), yeast extract (1%), $K_2HPO_4$ (0.05%) agar medium, and possessing light mouse gray aerial hyphae,[2] consistently produces fermentation broths exhibiting significant activity against Leukemia L-1210.

The active principle (NSC-113233) has been given the name mitomalcin (2).

The fermentation is most effectively carried out by first preparing a preform inoculum incubated for 24 hr at 28° on a reciprocating shaker.[3] Broth potency is optimized on

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[1] The parent culture, upon repeated slant transfer, loses its ability to produce the active antileukemic principle necessitating extensive microbiological examination of its susceptibility to natural variation.

[2] Electron microscopy reveals the unusual presence of hair-like projections on the spores.

[3] A high preform mycelium volume is generated when a medium consisting of 2% cornstarch and soybean meal, 0.5% yeast extract, 0.005% $MnCl_2$, $CuSO_4$, and $ZnSO_4$, 0.2% $CaCO_3$, and 0.25% NaCl is used.