Hormonal Control of Serum Albumin Synthesis (34080)

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Marked albuminuria has been noted in a number of laboratories investigating the effect of several functional pituitary tumors; *e.g.*, MtT/F4, MtT/W5, developed originally by Furth *et al.* (1–5). While this abnormal excretion of albumin in urine may have been related to a disturbance at the level of the kidney (6), it was also possible that the etiology resided in an excessive production of serum albumin by the liver. Previous studies have described the effect of MtT and its hormones on amino acid incorporation into trichloroacetic acid–insoluble material (cf. 3, 7, 8). This report deals specifically with albumin synthesis in rats with an abnormal hormonal pattern due to the presence of MtT implants.

Materials and Methods. Treatment of animals. Young male Fischer (F344) strain rats kept on a diet of Wayne Blox received subcutaneous implants of a Furth pituitary tumor MtT/F4, obtained from Dr. R. W. Bates (1, 2). The subcutaneous implant became palpable after approximately 4 weeks. Biochemical studies were carried out after 8–10 weeks, when the tumors weighed 5–8 g. Untreated rats of the same age served as controls.

Rate of albumin synthesis. Rats (weighing approximately 270 g) were injected with 10 μCi of a mixture of uniformly ^{14}C-labeled amino acids (New England Nuclear, Product No. 445). Blood was obtained from the tail 15, 30, and 45 min after injection. In preliminary experiments we established that albumin synthesis proceeded linearly for at least 90 min. The blood was drained into a heparinized spot plate and drawn into a syringe to measure its volume. Plasma was obtained by centrifugation of the whole blood for 3 min in a test tube using the Beckman-Spinco microfuge.

Separation of albumin by electrophoresis. Aliquots of 25 μl of plasma were applied on paper strips and electrophoresis was performed on a Beckman apparatus in a Michaelis buffer at pH 8.5 at 5 mA for 18 hr. The albumin band was localized by spraying a marker strip with bromphenol blue. After elution of the albumin band with 0.2 *N* NaOH, quantitation was obtained at 750 nm by the method of Lowry *et al.* as modified by Fiszer (9). Radioactivity was determined by scintillation counting in Bray's mixture of aliquots of the eluate and corrected for quenching (internal standard), background, and efficiency. Preliminary tests indicated that the albumin fraction obtained by electrophoresis did not carry free amino acids inasmuch as dialysis of the plasma did not alter the specific radioactivity of the albumin fraction. Autoradiographs of the paper strips after electrophoresis showed the bulk of the isotope was associated with the albumin region. There was some activity at and near the origin. In addition, in other experiments (10) it was established by immunoelectrophoresis that the band analyzed corresponded to that of rat plasma albumin.

Determination of plasma volume. At the end of the assay of albumin production (usually 45 or 60 min) 0.20 ml of 0.5% Evans blue solution (Warner Chilcott, General Diagnostics Division) was injected into the femoral vein or into the heart. Blood was collected 5 min later in a heparinized syringe. The plasma volume was calculated from the dilution of the dye (measured at 600 nm), with correction made from the amounts collected previously for the determination of the rate of albumin synthesis.

Preparation and determination of microsomal RNA. Whole livers or portions of livers weighing 8–10 g were homogenized with 4

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vol of a 0.25 *M* sucrose solution containing 0.05 *M* Tris buffer (pH 7.6), 0.005 *M* $MgCl_2$ and 0.025 *M* KCl. After a preliminary sedimentation at 20,000*g* for 15 min, a 25-ml portion of the supernatant fraction was centrifuged at 120,000*g* for 90 min. The resulting pellet of microsomes was taken up in 5 ml of Tris buffer and a 1-ml aliquot was treated with an equal volume of cold 10% trichloroacetic acid (TCA) and centrifuged. The residue was extracted twice with 4 vol of 5% TCA at 100° for 15 min. The combined extracts were adjusted to 10 ml with 5% TCA and RNA was determined on 0.1-ml aliquots by the orcinol reaction (11).

Determination of the free amino acid pool in liver. The supernatant soluble fraction, obtained above, was diluted to 25 ml with Tris buffer, and a 3-ml aliquot was deproteinized with tungstic acid. The clear supernatant fluid, adjusted to pH 6 with NaOH, was diluted to 10 ml with water. The total amino acids were determined at 520 nm on 50 μl-aliquots by the ninhydrin reaction (12). An additional 1-ml aliquot was used for radioactivity assay.

Results. The liver of rats bearing MtT were characteristically enlarged, usually more than threefold (Table I). Likewise, the plasma volume was considerably increased. However, the concentration of albumin in the plasma was decreased quite appreciably, and the hematocrit was lowered from 46–50% (in controls) to 35–41% (see 3).

The rate of albumin synthesis in the entire liver (completely linear for 60 min) of the MtT-bearing rats was more than twice that of the control rats, but the rate per gram of liver was lower. The livers of the tumor-bearing rats contained large amounts of microsomal RNA expressed as function of total weight or per gram. Thus, the rate of albumin synthesis per milligram of RNA was considerably lower in animals with MtT. The rate values described were corrected for the specific radioactivity of the pool of free amino acids in the liver. The size of the pool in the tumor-bearing animals was about twice that of the controls.

Discussion. Rats with a transplantable functional pituitary tumor exhibit a severely altered picture of albumin synthesis and excretion. The exact mechanism of loss of albumin in the urine is not understood but it appears that a compensatory rise in the synthesis of albumin occurs in the liver. It is of interest that rats bearing MtT and fed a liver carcinogen do not exhibit a marked proteinuria, and have smaller kidneys although a diabetes-like syndrome characterized by polphagia, polydipsia, and glucosuria is observed (4). Preliminary tests indicate that albumin synthesis is lower in carcinogen-fed rats.

In MtT rats, two possibilities may account for the increased synthesis of albumin in liver: (1) the ribosomes instrumental in the

TABLE I. Effect of a Functional Pituitary Tumor, MtT/F4, on Albumin Synthesis.

	Rats with MtT	Controls	Ratio MtT/controls
Body weights	270 ± 5 g	289 ± 24 g	0.94
Ratio liver/body weights	0.125 ± 0.0006	0.0384 ± 0.0001	3.25
Plasma volume/g body weight	74.2 ± 2.2 μl	37.0 ± 1.8 μl	2.0
Albumin concentration/ml plasma	18.5 ± 3.6 mg	28.5 ± 3.6 mg	0.65
Radioactivity appearing in plasma albualbumin per min	8830 ± 33 cpm	6050 ± 249 cpm	
Specific radioactivity of free amino acids in liver	2190 ± 510 cpm/μmole	2880 ± 80 cpm/μmole	
Rate albumin synthesis/liver	4.26 ± 0.98	2.10 ± 0.03	2.0
Rate albumin synthesis/g liver	0.13 ± 0.03	0.19 ± 0.018	0.67
Rate albumin synthesis/mg microsomal RNA	0.054 ± 0.009	0.15 ± 0.013	0.36
Microsomal RNA/g liver	2.33 ± 0.15 mg	1.25 ± 0.01 mg	1.9
Total liver microsomal RNA	78.2 ± 4.4 mg	13.9 ± 1.0 mg	5.6

synthesis could be more efficient, or (2) there could be more producing units. The results show that the latter scheme appears to be the mechanism involved. The liver and kidneys are considerably enlarged, perhaps as a result of functional demands. The enlarged liver contains almost twice the amount of ribosomal RNA. Nonetheless, each RNA unit seems less effective inasmuch as on a weight basis, RNA from MtT rats yields only 35% of the albumin synthesized by RNA of control rats. Thus, the total rate of production may be increased only because of the considerable enlargement of the liver, and hence of the number of producing units.

Of interest also is the apparent dilution of the blood as regards both concentration of plasma albumin and red cell volume, which may be due in part to the increased water intake. However, there is also a marked polyuria (3, 4) in the MtT-bearing rats. The decrease in red blood cell volume may be also due to tumor growth.

The decreased unit efficiency of ribosomal RNA may not be limited to that fraction of the endoplasmic reticulum. Indeed, we have demonstrated that compared to controls, rats with MtT excreted larger amounts of *N*-hydroxy-*N*-2-fluorenylacetamide in the urine as a glucosiduronic acid after a single dose or upon chronic treatment (4, 13). This finding was generalized subsequently by Wilson (14, 15), who observed that the capability to metabolize drugs by a number of pathways was greatly reduced in rats with MtT. Such reduced conversion of drugs occurred even in adrenalectomized rats suggesting a direct effect of hormonal factors from MtT on the liver (16). These results are somewhat at variance from those dealing with protein synthesis where the MtT effect was abolished by adrenalectomy and restored by select adrenal hormones supplemented with salt solution (3). It would appear from all these studies that hormonal factors greatly affect the smooth and the rough endoplasmic reticulum of liver directly or indirectly (5, 17, 18).

Summary. Eight to ten weeks after implantation of a functional pituitary tumor, MtT/F4 (Furth), into young male Fischer strain rats the following ratios were increased in relation to controls: Liver weight and plasma volume per gram body weight, the rate of albumin synthesis and the total microsomal RNA per liver, and the microsomal RNA per gram liver. However, the albumin concentration per milliliter plasma, the rate of albumin synthesis per gram liver or per milligram microsomal RNA were decreased. It is concluded that in rats with MtT the ribosomal units are less effective in albumin synthesis, but that the total amount of albumin synthesized is larger because of the considerable increase in liver size and total microsomal RNA. The effect of MtT on the function of the endoplasmic reticulum is discussed.

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Received April 7, 1969. P.S.E.B.M., 1969, Vol. 131.