as the weight of the pituitary, thyroid and thymus undergo a gradual decrease as the hormone dosage is increased. The seminal vesicle, prostate and epididymis increase in size with increasing dosage. On the other hand, the inhibitory action of testosterone on the adrenal and testis has a definite optimum at the 0.5 to 1.0 mg daily dose level, inasmuch as smaller doses cause less pronounced atrophy and larger amounts may actually stimulate the growth of these glands. The histological changes which accompany these variations in gross weight have been described. We conclude that many of the apparent contradictions concerning the morphogenetic actions of androgens—and perhaps also of other steroid hormones—are due to the fact that depending on the dose given, these compounds may exert diametrically opposite actions on certain organs.

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Riboflavin Content of Blood and Muscle in Normal and in Malnourished Humans.*

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A number of workers have reported significant decreases in the riboflavin content of tissues from experimental animals maintained on a riboflavin deficient diet.[1-6] The resultant conclusions, however, cannot safely be applied to man.

* University of Cincinnati Studies in Nutrition at the Hillman Hospital, Birmingham, Alabama. These studies were aided by grants from the John and Mary R. Markle Foundation and the Wisconsin Alumni Research Foundation.

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In this study we have made a comparison of the blood and muscle riboflavin values in a group of normal subjects and in a group of malnourished persons selected from the Nutrition Clinic, Hillman Hospital, Birmingham, Alabama.

Blood Studies. One blood sample was taken before therapy from each of 35 patients with varying degrees of riboflavin deficiency and from 30 members of the laboratory staff. A diagnosis of riboflavin deficiency in these patients was based on the presence of cheilosis and conjunctivitis which, in each instance, were relieved by the oral administration of riboflavin. Five ml of blood were obtained by venepuncture and potassium oxalate was used as the anticoagulant. Precautions were taken to avoid exposure to light and the blood was stored in the refrigerator occasionally for a 2-week period as no diminution in the riboflavin content due to storage under these conditions was ever observed.

The riboflavin content of blood was determined according to the microbiological method of Snell and Strong.[7] In this method, which is based upon the fact that riboflavin is essential for the growth of a specific strain of *Lactobacillus casei*, the growth of the organism, as measured by the acid production, is proportional to the amount of riboflavin added to a riboflavin-deficient medium. The reliability of this method for the determination of both the free and the combined forms of riboflavin in a number of biological fluids has been established definitely by a variety of tests.[7, 8, 9] The blood was hemolyzed immediately before the analysis and aliquots of hemolyzed blood equivalent to 0.1 and 0.2 ml of whole blood were added to assay tubes in duplicate. The addition of larger amounts of hemolyzed blood yielded lower values of riboflavin when calculated per ml of the original blood, a phenomenon which suggested the presence of inhibiting substances in appreciable quantities when amounts of hemolyzed blood equivalent to more than 0.2 ml of whole blood were added. The results obtained when 0.2 ml of whole blood or less were used per assay tube were considered accurate since recovery experiments at these concentrations proved to be satisfactory.

The blood riboflavin values of the 20 normal subjects ranged between 0.35 and 0.45 μg per ml with an average value of 0.42 μg per ml. In the blood of the patients with clinical evidence of riboflavin

[7] Snell, E. E., and Strong, F. M., *Ind. and Eng. Chem.* (Analyt. Ed.), 1939, **11**, 346.

[8] Feeney, R. E., and Strong, F. M., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, 1940, **133**, 31.

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deficiency, the range of values was the same as in the control group with an average value of 0.40 μg per ml. There was no indication that the concentration of riboflavin in the blood decreased as the deficiency lesions became more severe.

Muscle Studies. Muscle specimens were obtained by biopsy from 30 patients who were chosen because they represented a group with varying degrees of pellagra and interrelated vitamin deficiencies. Our prime interest in these cases was the study of the coenzyme I content of muscle as related to the degree of nicotinic acid deficiency. This group, although presumably riboflavin deficient to some degree, showed no striking clinical manifestations of this specific deficiency. Muscle specimens obtained by biopsy from 9 patients of normal nutrition who were undergoing a herniorrhaphy at the Hillman Hospital served as a control group.

The section of the quadriceps femoris muscle, removed at biopsy, was immediately frozen on carbon dioxide snow. A portion of this frozen tissue was kept for moisture determinations and another sample was ground to a fine powder in a mortar which was kept at the temperature of the carbon dioxide. No significant variation in moisture content was observed. The finely pulverized tissue (0.5-1.0 g) was weighed on a cold watch glass and washed with approximately 5 cc of hot water into 25 cc of boiling water. The extract was boiled for 3 minutes and cooled immediately. These precautions were taken in order to preserve the coenzyme I content of the tissues and were probably not necessary in the riboflavin determination. The coenzyme I content was determined in an aliquot of this extract[10] and the remainder was stored in the refrigerator under toluene and later used for the determination of riboflavin according to the microbiological method of Snell and Strong.

The riboflavin contents of the muscles from the control group ranged between 2.2 and 3.5 μg per gram of fresh weight, with an average value of 2.9 μg per gram. The values for the 30 patients in the malnourished group also lay within the same range with an average value of 2.8 μg of riboflavin per gram of muscle.

Summary and Conclusions. 1. The blood riboflavin values of 20 normal subjects ranged between 0.35 and 0.45 μg per ml, with an average value of 0.42 μg per ml. The same range of riboflavin values was found in a group of 35 patients with varying degrees of riboflavin deficiency. The average value for this group was 0.40 μg of riboflavin per ml.

2. The muscle riboflavin values of 9 control subjects ranged

[10] Axelrod, A. E., Spies, T. D., and Elvehjem, C. A., *J. Biol. Chem.*, in press.

between 2.2 and 3.5 μg per gram of fresh weight with an average value of 2.9 μg per gram. The muscle riboflavin values for 30 pellagrins lay within the same range with an average value of 2.8 μg per gram of fresh muscle.

3. From these results we have concluded that a determination of blood and muscle riboflavin values is of little significance in the evaluation of a state of riboflavin deficiency in man.

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Assay of Secretin.

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Recently a secretin preparation designed for human use was developed and tested clinically in normal individuals and in patients with pancreatic and biliary tract disease.[1, 2, 3] Prepared by an unpublished method, this product is now manufactured and sold under the name *Pancreotest* by the Astra Laboratories, Södertälje, Sweden. Through the courtesy of Messrs. Astra, a sample of Pancreotest was made available to us. This particular material had been produced recently (May 1, 1940) and was standardized by the manufacturer against the crystalline secretin of Hammarsten, *et al.*[4] Having this standardized product we could evaluate its potency in relation to that of our own secretin preparations and investigate an apparent discrepancy in the expression of secretin units. Assays were conducted on 5 dogs by the technic previously described.[5]

Results. Pancreotest was found to be free of vasodilator substances when injected intravenously in amounts up to 10 mg. Weight for weight it was about half as potent as our standard SI preparation in stimulating the pancreas to secrete.[5] The data on the 5 dogs are given in Table I.

Discussion. As is evident from the table, a given amount of Pan-

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