represent an absorption of 141.6 mg of testosterone. Previous studies have shown that, when administered intramuscularly in solution in sesame oil, the absorption is very rapid and consequently a considerable amount of the hormone is excreted before it can produce any effect.

Summary and Conclusions. Eight women with regular menstrual periods and normal cyclic estrogen and progesterone production, as evidenced by the finding of typical secretory endometria premenstrually, were treated with androgens (methyl testosterone by mouth, testosterone propionate by injection, and testosterone and testosterone propionate by implantation) in order to determine the effect of the androgens upon excretion of SPG. The results indicate that doses of androgens which have been found, in previous studies, to be suppressive (*i. e.*, to cause suppression of pituitary gonadotropic hormone excretion; inhibition of ovulation and menstruation), when administered early in the cycle, result in failure of SPG to appear in the urine during the current cycle and lead to suppression of the next expected menstrual period. In one patient (Case No. 6), 1175 mg of testosterone propionate, administered after the 9th day of the cycle, did not appear to appreciably affect the excretion of the pregnandiol complex during the current cycle.

These results are interpreted as indicating that if sufficient androgens are administered early in the cycle, before the pituitary gonadotropin has been secreted or had time to exert its effect on the ovary, progesterone production is suppressed, resulting in absence of pregnandiol from the urine during the current cycle. That absence of SPG from the urine is due to failure of progesterone formation and not to interference with the conversion of progesterone to the pregnandiol complex is proven by the absence of corpora lutea from the ovaries in the patients with no pregnandiol excretion.

13205

Some Aspects of Metabolism Following Parenteral Administration of Casein Hydrolysate.

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During the process of digestion of proteins, amino acids are liberated and pass unchanged into the portal circulation. A portion of these amino acids is utilized as a source of energy while another is used by the body for the formation of many characteristic tissue proteins.[1] According to Rose the indispensable amino acids are as follows: lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine. (The indispensability of arginine is questioned.) Of these amino acids l-tryptophane, l-histidine, l-phenylalanine, and l-methionine can be replaced for growth purposes by their optical isomers.

This paper deals with: (a) A method for the preparation of a hydrolysate of casein suitable for parenteral administration to humans; and (b) its effect on urea-N and amino acid-N of the blood of fasting rabbits.

Methods. Blood amino acid-nitrogen was determined by Sahyun's methods,[2] and blood urea nitrogen by Wrenn's.[3]

Experimental. The effects of sulfuric acid and of barium hydroxide were studied on each of the amino acids listed in Table I. A 100-mg sample of each amino acid was treated with 5 N sulfuric acid and 40% commercial barium hydroxide respectively. The samples were autoclaved at 20 lb steam pressure for 12 hours. Each sample was then made alkaline to phenolphthalein, filtered, and aliquot samples of the filtrate immersed in a boiling water bath to drive off any ammonia that might have been formed. Nitrogen was then determined. The results are given in Table I.

Of the amino acids studied cystine and arginine suffered either loss or destruction by treatment with barium hydroxide. A portion of lysine was destroyed, while tryptophane was completely destroyed by treatment with sulfuric acid.

Procedure A—Acid Hydrolysis. Into a 20-liter Pyrex bottle

TABLE I.
Effect of Autoclaving for 12 hr at 20 lb Steam Pressure of Certain Amino Acids in the Presence of 5 N Sulfuric Acid and 40% Barium Hydroxide. Mg Nitrogen per Sample.

		Autoclaving with	
Amino acid	Amount, mg	Barium hydroxide, mg	Sulfuric acid, mg
Lysine	6.8	6.5	4.8
Tryptophane	13.7	11.2	5.6
Arginine	26.0	11.0	No change

Histidine, phenylalanine, leucine and valine were not affected by autoclaving with either barium hydroxide or sulfuric acid.

1 Rose, W. C., *Physiol. Rev.*, 1938, **18**, 109.
2 Sahyun, M., *J. Lab. Clin. Med.*, 1939, **24**, 548.
3 Wrenn, H., *J. Lab. Clin. Med.*, 1937, **22**, 1040.

10 liters of 5 N sulfuric acid were introduced, then 2 kg of casein were added slowly while stirring. The bottle was then covered with a piece of cheesecloth and placed in an autoclave. Steam pressure was gradually increased until it reached 20 lb per square inch. Autoclaving was continued for about 16 hours. The content of the bottle was allowed to cool over night, then calcium oxide (slacked lime) was added until the reaction was decidedly alkaline to phenolphthalein. Distilled water was then added until the volume was about 30 liters. The hydrolysate was allowed to stand over night until the calcium sulfate had settled, then the clear supernatant liquid was siphoned off. The calcium sulfate precipitate was filtered, washed with hot distilled water, and the washings were added to the filtrate. To remove ammonia the alkaline filtrate was next concentrated in vacuum to about 10 liters. The ammonia-free filtrate was next saturated with carbon dioxide and a considerable portion of the excess calcium was removed as calcium carbonate. This was filtered off and oxalic acid was added to the clear filtrate to precipitate out most of the remaining calcium. The liquid was heated, filtered, and to the clear filtrate barium hydroxide was added to precipitate the last traces of sulfate present in the hydrolysate. The addition of barium hydroxide was carefully controlled so that a filtered sample gave but a slight test for sulfate, thus the absence of barium was assured. As a rule, the amount of sulfate did not exceed 5 to 10 mg calculated as sulfuric acid per 100 cc. Nitrogen was next determined. The final preparation was either concentrated in vacuum or diluted with pyrogen-free doubly-distilled water to obtain a desired concentration. The acidity was adjusted to about pH 5. l-Tryptophane and cystine were added to the extent of 1.0% of the total amino acids present calculated on the basis that a solution of the mixed amino acids containing 2.1% nitrogen would yield about 15% amino acids. (From this concentrate suitable dilutions of 5% and 10% were made for clinical and animal experimentation.) This mixture was filtered through a clarifying pad, sterilized by passing it through a sterile Berkefeld filter, and stored in sterile bottles in the cold at about 4°C.

Procedure B—Acid-Base Hydrolysis. One kg of casein was hydrolyzed in 5 N sulfuric acid by autoclaving for about 16 hours as described above; simultaneously another one kg was hydrolyzed in 5 liters of 40% barium hydroxide. Hydrolysis was judged complete when samples yielded a negative biuret test. Then, while stirring, the sulfuric acid hydrolysate was slowly added to the barium hydrolysate. The reaction of the mixture was next made alkaline with barium hydroxide using phenolphthalein as an indicator. Ammonia was removed by concentration of the mixture in vacuum to about half of the original volume. The excess of barium was removed by the addition of sulfuric acid so that a filtered sample gave but a faint sulfate test. This material was filtered and about 6 to 8 g of oxalic acid was added to the clear filtrate to precipitate any excess calcium found in the solution from the hydrolyzed casein. The preparation was adjusted so that the final nitrogen content was 2.1%, which would yield about 15% amino acids. (From this concentrate suitable dilutions were made for clinical and animal experimentation.) This mixture of amino acids was termed "acid-base hydrolysate" of casein. It is to be remembered, however, that about one-half of the amino acids in the final preparation consisted of racemized amino acids.

Experiment on Rabbits. The following solutions were made in doubly distilled water: (1) A 10% solution of glycine, (2) a 10% solution of acid hydrolysate "A" with added tryptophane and cystine, and (3) a 10% solution of acid-base hydrolysate "B". Ten cc per kg body weight of each of the 3 preparations were subcutaneously administered into each of a group of 10 rabbits that had fasted for 24 hours. Samples of blood were withdrawn at 0, 1.5, 3, 5, and 7 hour intervals respectively. Urea-N and amino acid-N were determined. The averages of the results for this experiment are reported in Figs. 1 and 2.

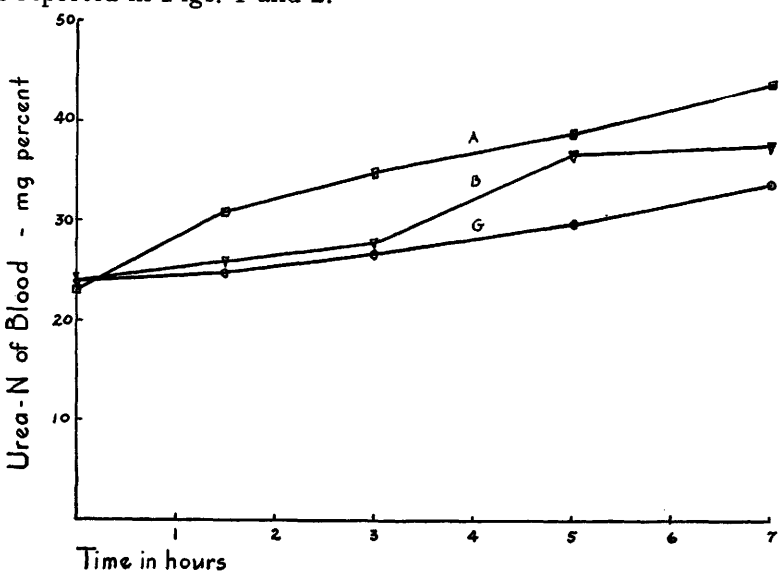

FIG. 1.
Blood Urea Nitrogen.

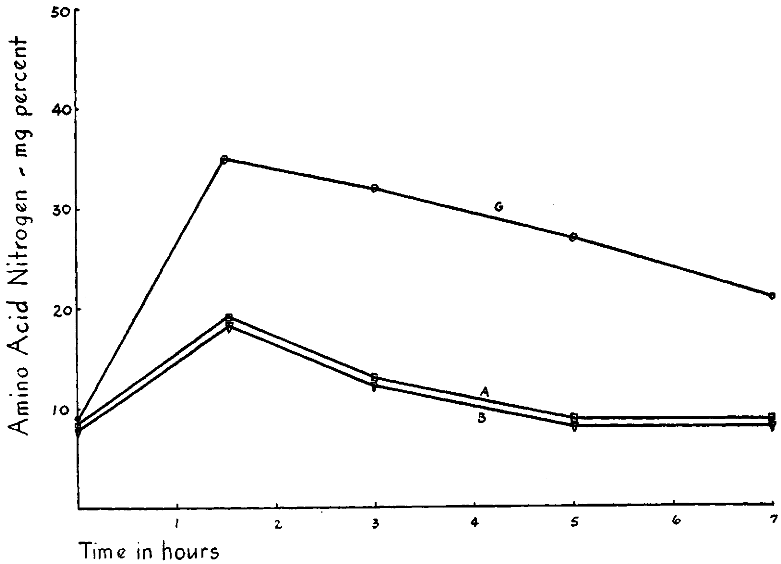

FIG. 2.

Blood Amino Acid Nitrogen.

Following the effects of the subcutaneous administration into fasting rabbits of 10 cc of a 10% solution per kg body weight of : (A) Acid hydrolysate of casein with added tryptophane and cystine, (B) Acid-base hydrolysate, and (G) Glycine. Each curve represents the averages of the results obtained on 10 rabbits.

Discussion. The data presented on the effect of glycine on amino acid nitrogen, and urea nitrogen of blood are in harmony with the earlier work of Lewis and Izume,[4, 5] and of Blatherwick, Sahyun, and Hill.[6] The experiment on the administration of glycine was performed to serve as a basis of comparison between the administration of a single amino acid and a mixture of amino acids. The results obtained indicated very clearly that the administration of a mixture of amino acids caused a more rapid deaminization as was shown by the faster rate of urea formation and smaller accumulation of amino acid nitrogen in the blood as compared with the figures obtained following the administration of glycine. The considerable increase in blood amino acid nitrogen observed (Fig. 2) following the injection of glycine may be attributed in part or *in toto* to the fact that the dose of glycine was about twice as great in respect to NH_2-N as doses of amino acid mixtures because of the higher average of molecular weight of the latter.

Following the administration of the acid-base hydrolysate the results obtained showed a less marked rate of deaminization as com-

[4] Lewis, H. B., and Izume, S., *J. Biol. Chem.*, 1926, **71**, 33.

[5] Izume, S., and Lewis, H. B., *J. Biol. Chem.*, 1926, **71**, 51.

[6] Blatherwick, N. R., Sahyun, M., and Hill, E., *J. Biol. Chem.*, 1927, **75**, 671.

pared with the result obtained following the administration of the acid hydrolysate to which tryptophane and cystine had been added. This difference in action between the two types of casein hydrolysate used in this experiment may be explained on the basis that in the acid-base hydrolysate almost half of the amino acids administered were in the racemic form due to the procedure employed in hydrolyzing 50% of the casein with barium hydroxide.

Summary. A method for the preparation of a casein hydrolysate suitable for parenteral administration is described. Data are presented showing greater utilization and deaminization following the parenteral administration into fasting rabbits of an acid hydrolysate of casein to which l-tryptophane and l-cystine had been added, than following the administration of either glycine or partially racemized amino acids of an acid-base hydrolysate of casein.

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Enhancing Effect of Nicotinic Acid and Cysteine Hydrochloride on Growth of *Leptospira icterohaemorrhagiae*.*

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While carrying out various studies on *L. icterohaemorrhagiae,* the difficulty of growing these organisms in quantities suitable for use as antigen in the agglutination or complement fixation tests constantly presented itself. Likewise, some difficulty had been encountered in perpetuating strains of leptospira in culture media, since they died for no apparent reason, and batches of media prepared identical to previous ones would often not sustain the growth of these organisms.

In an attempt partially to resolve these difficulties, the effect of the addition of various amino acids and nicotinic acid to a modified Noguchi semi-solid medium was tested.

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