mals, while Block(7,8) demonstrated that under normal conditions the *in vitro* rat liver produced 32-65 μM % GSH per hour. The rate of recovery in the present experiments was somewhat slower than that which would be predicted from calculations based on these figures, even if it is assumed that recovery did not begin until normal body temperature had been obtained.

From a knowledge of the difference in the response of fasting and nonfasting animals, it could be reasoned that the available energy may play an important role in regeneration of NPSH. The stimulating effect of added succinate, fumarate, or malonate on the *in vitro* synthesis of GSH confirms the impression that the formation of the NPSH (GSH at least) is associated with energy yielding reactions (7). In these stressed animals the adenosine triphosphate (ATP) would very probably have been low, and low ATP has been shown to depress GSH production(7).

Summary. 1. Adult female Sprague-Dawley rats were exposed to cold and restraint to effect a lowering of concentration of the total nonprotein sulfhydryl compounds (NPSH) in the liver. At the termination of this exposure the animals were divided into 2 categories (fasting and nonfasting) and were permitted to recover under normal laboratory conditions. During the recovery period animals were sacrificed at intervals to trace the rate of regeneration of the liver and kidney NPSH. 2. In neither the fasting nor the nonfasting categories was there a significant regeneration of the liver NPSH in 8 hours. In the nonfasting recovery was virtually complete in 16 hours and complete in 24 hours. In the fasting animals recovery was slower in beginning and was not completed by 24 hours. The rate of loss of NPSH from the liver was considerably greater, in these experiments, than the rate of recovery. 3. Exposure to cold and restraint appeared to lower the kidney NPSH. Recovery was completed in fasting and nonfasting animals in less than 8 hours, with a transient overcompensation in both categories.

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Free Amino Acids and Glutathione of Normal and Syphilitic Rabbit Testes.* (21248)

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The successful *in vitro* cultivation of pathogenic *Treponema pallidum* remains one of the major unsolved problems in syphilology. In seeking solutions to the problem the metabolic requirements of avirulent strains of spirochetes purported to be *T. pallidum* were investigated. A chemically defined medium for the cultured Reiter strain of *T. pallidum* was found(1) but the results have not been of value in the attempted cultivation of the pathogenic organism. Conditions favoring the *in vitro* survival of the pathogenic (usually Nichols) strain of *T. pallidum* led to the development of the treponemal immobilization test of Nelson and Mayer(2). None of the

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suggested media have resulted in significant *in vitro* multiplication of pathogenic *T. pallidum*. Work from this laboratory(3) has indicated that there may be an early disassociation of virulence and motility with the result that organisms may lose their virulence in a period of 2 to 4 days even though their *in vitro* motility persists as long as 10 to 14 days. In view of this, it seems worthwhile to seek other approaches to the problem.

Pathogenic *T. pallidum*, like many other microorganisms, demonstrates a preference for certain tissues. In the rabbit, regardless of the route of inoculation, a syphilitic orchitis occurs. Thus, these tissues are usually employed as the site of inoculation. It was felt advisable to begin a comparative study of the biochemistry of these tissues in the hope that comparisons between susceptible and non-susceptible tissues, and comparisons between infected and non-infected susceptible tissues might suggest factors necessary for the growth of the organisms. This paper is concerned with changes in the free amino acids, glutathione, and solid content of normal and syphilitic rabbit testes.

Experimental. Free amino acids of testes. Preparation of protein-free amino acid concentrates. The initial steps were performed in a cold room at 5°C. Fifty-two g of testes (from 6 to 15 rabbits) were homogenized in a precooled Waring blendor with 104 ml (2 vol.) of cold distilled water. Duplicate 3 ml samples were removed for the estimation of solid content as determined by weight after drying for 24 hours at 100°C. To the balance of the homogenate 560 ml of cold absolute ethanol were added and the mixture stirred for one minute, filtered at room temperature with refiltration until the filtrate was clear. The precipitate was then washed with 50 ml of 80% ethanol. The combined filtrates were evaporated to dryness in vacuum at 30°C. The residue was extracted with successive 10, 5, and 3 ml portions of 10% isopropyl alcohol in water. The resultant suspension was then intermittently shaken for a 30-minute period with 6 g of the strongly acidic exchange resin, Amberlite IR-105 (H). The resin was allowed to settle, and the supernatant, containing a flocculent precipitate of proteins and other large molecules was decanted and discarded. The Amberlite was then washed 10 times with successive 15-ml portions of distilled water. These supernatants were discarded. The amino acids were then recovered from the Amberlite resin by shaking the resin for 15 minutes with 5 ml of 5 N ammonium hydroxide. The supernatant and 5 successive washes with 10 ml of distilled water were filtered by gravity. The combined filtrates were then evaporated to dryness in vacuum at 30°C and the residue was redissolved in 2.5 ml of 10% isopropyl alcohol in water. Aliquots of this solution were employed for filter paper chromatography. These amino acid concentrates could be stored for long periods of time at 5°C. Some of the amino acids crystallized from solution during prolonged storage, but on warming of the tube they promptly redissolved. *Chromatography of free amino acids.* The 2-dimensional method of Levy and Chung(4) was employed with certain modifications. One or 2 μl of the protein-free amino acid concentrate were applied at a corner 3 inches from each edge of a 22.5 x 18.5 inch sheet of Whatman No. 52 paper. The applications were slowly dried on the paper with a hair drier. The chromatograms were run at room temperature in a Chromatocab. A butanol-acetic acid-water (4:1:5) mixture was used for the development in the first dimension. The descending method was used. For the second dimension, the developing mixture consisted of 120 g of redistilled phenol, 120 g of redistilled m-cresol, 34 ml of 0.15 M pH 9.4 sodium borate buffer, and 50 mg of ascorbic acid in 2.5 ml of distilled water. The amino acid spots were identified with the ninhydrin reagent of Levy and Chung, dissolved in methanol rather than ethanol. The reagent was sprayed on the paper with a glass sprayer. A Welch Densichron was employed to estimate the density of the amino acid spots. The area under the resultant curve was a function of the concentration of the given amino acid, and this area could be used to estimate the quantity of amino acid present. In the calibration of the quantitative procedure, amino acids were run singly and in mixtures to obtain planimeter values of the density readings within the range

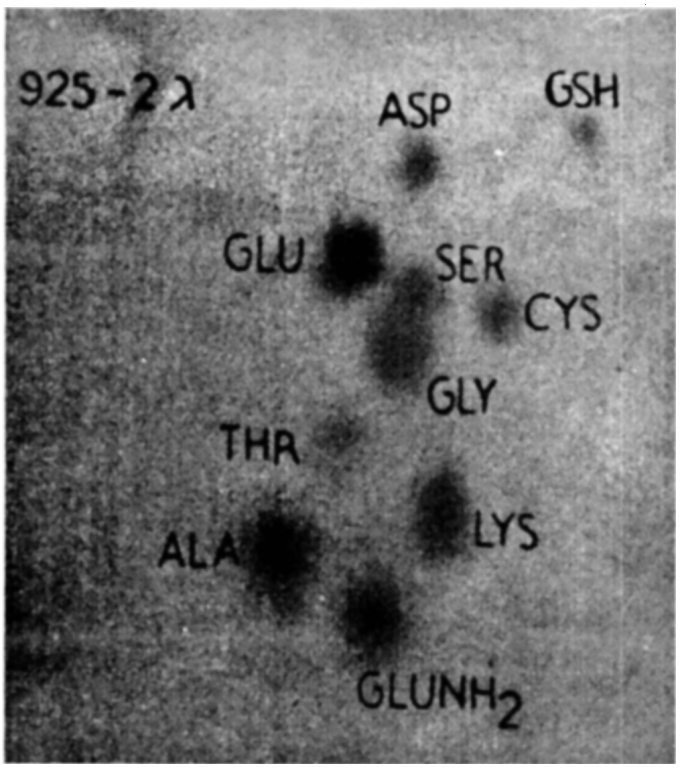

FIG. 1. Chromatogram of a protein-free Amberlite treated, amino acid concentrate of a syphilitic testes extract. The spots are those of 9 amino acids and of oxidized glutathione.

to be employed for quantitative work on the testes. A satisfactory calibration curve could not be obtained for the cystine standard and the values here reported are relative only and do not represent absolute values. These relative curves were based upon the densities of the alanine calibration curve. In early attempts to separate these amino acids we were unable to effect a separation of the serine, glycine, and cystine from the large quantities of reduced glutathione. After the Amberlite-ammonium adsorption and elution procedure was adopted no further difficulty was encountered since all of the glutathione was oxidized in the alkaline medium without being hydrolyzed. The R_f of the oxidized gutathione is extremely low and it does not interfere with the determination of the amino acids. Under the conditions of the present method nearly all of the amino acids gave a bluish purple color with ninhydrin. The glycine and the oxidized glutathione gave a reddish purple color and the asparagine, present only in trace amounts, gave a yellow color. The absolute quantities of amino acids measured varied from 0.7 μg (glutamine) to 8.3 μg (glutamic acid). These represent the extremes in the 8 pools under study. *Reduced glutathione.* The reduced glutathione was determined in protein-free filtrates(5). A weighed quantity of freshly excised tissue (approximately 400 mg) was frozen in solid carbon dioxide and then homogenized in 3% metaphosphoric acid and one ml of cold distilled water. The Klett-Summerson colorimeter with green filter No. 54 was used to measure the intensity of the color produced by the addition of sodium nitroprusside. Within the range of our experiments (0.01-0.1 mg) the intensity of the color followed Beer's Law.

Results. Fig. 1 demonstrates a typical chromatogram obtained from a 2 μl sample of the amino acid concentrate from syphilitic testes. The location of aspartic acid, glutamic acid, serine, glycine, cystine, alanine, lysine, and glutamine are shown. In addition a trace of threonine is shown by the lighter spot. Not included in Fig. 1 are the more mobile

TABLE I. Free Amino Acid Content of Testes (mg/100 g on Dry Basis).

	Solids, %	Asp	Glu	Ser	Gly	Lys	$GluNH_2$	Ala	Cys
				Normal group					
	22.30	7.5	50.1	17.7	62.0	26.6	14.4	12.3	15.4
	24.15	5.4	65.8	9.9	52.6	45.0	21.6	16.9	15.8
	19.90	11.2	66.4	14.2	33.3	24.4	22.6	17.2	18.0
				Syphilitic group					
(8)*	20.95	12.4	58.3	13.2	48.7	43.5	7.8	17.3	17.7
(13)	14.10	6.2	48.5	13.3	39.3	34.0	Trace	14.6	13.1
(27 to 82)	24.80	7.7	84.4	13.2	40.5	23.4	18.9	20.5	15.7
(80 to 104)	37.87	5.0	27.4	6.8	17.8	16.2	12.6	8.6	10.8
(156 to 180)	32.50	6.5	47.8	16.5	24.8	29.5	27.8	17.9	7.5

* Figures in parentheses indicate period of infection in days.

amino acids, methionine, leucine (or isoleucine), and asparagine (which gave a yellow color with ninhydrin) which are also present in trace amounts. The spot close to the point of application is that of oxidized glutathione. All of the glutathione is oxidized by the alkaline elution method. Adsorption on and subsequent elution from the strongly acidic Amberlite IR-105 (H) effected the removal of proteins and other large molecules and the partition of several amino acids after oxidation of the reduced glutathione.

In Table I is seen that during the most virulent stage of infection (8 to 13 days) there is a significant decrease in the free glutamine content of testes. There is a drop in the solid content of testes at 10 to 15 days of infection. The other free amino acid values, however, are not appreciably different from the normal values as observed during the experimental period of 8 days to 180 days of infection.

On a wet basis the reduced glutathione content of syphilitic rabbit testes average 65% of the normal value. On dry basis it is 74% of the normal value. The normal group's dry weight was 15.0-28.3%, the average being 21.9%. The average glutathione values, using 50 normal rabbits in 17 experiments, were found to be 95.1 mg on wet basis (highest 116, lowest 68) and 453.1 mg on dry basis (highest 713, lowest 263). In the syphilitic group (2-180 days incubation time), employing 45 rabbits in 14 experiments, the average was 62.0 mg on wet basis (highest 85, lowest 37), and 335.5 mg on dry basis (highest 509, lowest 126). All figures are per 100 g tissue. The dry weight was 10.8-38.6%, the average being 20.46%. The decreased reduced glutathione content of testes in syphilis is noteworthy. Recently Brückmann and Wertheimer(6) reported the following reduced glutathione values for normal rat organs: Pancreas, 60; kidney, 109; liver, 176 mg/100 g wet tissue. Our value of 95.1 mg (on wet basis) for normal rabbit testes is well within the range of those of rat kidney and rat liver.

Summary. A method for separating the free amino acids from proteins and other large molecules, and from interfering reduced glutathione has been devised. The strongly acidic exchange resin Amberlite IR-105 (H) has been employed for the adsorption of the free amino acids and ammonium hydroxide for their elution. The ammonia was removed *in vacuo*. The final purified concentrates were put in 10% isopropyl alcohol and subjected to 2-dimensional filter paper chromatography. The ninhydrin developed amino acid spots were measured by the densitometric method. The major amino acid components present in normal rabbit testes were aspartic acid, glutamic acid, serine, glycine, lysine, cystine, alanine, and glutamine. There were also present a trace of threonine, methionine, asparagine, and leucine (or isoleucine). In syphilis, during the most virulent stage of infection, there is a significant decrease in the free glutamine content of testes. Reduced glutathione, as determined by the quantitative nitroprusside test on protein free filtrates, was

found to be distinctly decreased in syphilitic testes.

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Effect of Aminopterin on Incorporation of C^{14} Formate into the 2 and 8 Carbons of Guanine.* (21249)

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Whether formate enters the 2 and 8 position of the purine ring(1) via the same immediate precursor or cofactor is not known. Its entry into the 2 position appears to require its prior incorporation into a reduced folic acid derivative in the presence of ATP (2). When aminopterin is injected into an animal this step may be partially blocked and it is possible that C^{14} formate incorporation might be so altered that the specific activity of the 2 carbon of purine would be different from that of the 8 carbon. This might occur if the immediate C^{14} precursors of the 2 and 8 carbons were different and the antimetabolite changed their pool sizes, or if a purine intermediate such as 5-amino-4-imidazolecarboxamide ribotide accumulated. However, in this study the C-2/C-8 ratio of C^{14} formate incorporation into rat nucleic acid guanine was consistently very close to 1 and did not change in the presence of aminopterin.

Methods. Guanine degraded in the following experiments was obtained from the nucleic acids of adult rats used in studies previously reported(3). All animals received C^{14} sodium formate,† 3.6 x 10^7 cts/min/kg by intraperitoneal route, while some of the animals received simultaneously aminopterin, 50 mg/kg. All animals were sacrificed after 24 hours, and the sodium nucleate from different tissues obtained by hot 10% sodium chloride extraction and ethanol precipitation. In control experiments the following organs were employed: 1, total viscera; 2, total viscera plus added small intestine. In the aminopterin experiments total viscera plus intestine were used in No. 3, while small intestine was used in No. 4. 750 mg samples of sodium nucleate were refluxed in 50 ml of 0.5 N H_2SO_4 for 2 hours, filtered, and to the filtrate 50 ml of 10% $AgNO_3$ was added. The purine silver salt was washed, decomposed with HCl at 100° and treated with norite. After filtering, the volume was reduced to 7 ml and guanine crystallized out on neutralization. This was recrystallized from 2 N H_2SO_4. 10 mg of guanine sulfate in 1 ml of 4 N H_2SO_4 was converted to xanthine by addition of 30 mg of $NaNO_2$ in 0.7 ml of water at 75° over a 10-minute period. After cooling at 2°C overnight, the xanthine which crystallized was collected, and converted to uric acid by means of xanthine oxidase(4). The conversion was estimated spectrophotometrically(5). 84 mg of carrier uric acid was added as the lithium salt and uric acid was precipitated on acidification. This was repeated, and the resulting

* The radioactive isotope used in these studies was obtained on allocation from the Atomic Energy Commission.

† Sodium formate 0.28 mM/mc. dissolved in 0.775 ml of dilute alkali.