TABLE III.

	10 hrs HS	24 hrs HS	24 hrs cont.	48 hr HS	48 hr cont.
% mitosis	.19	.39	.61	.43	.33

mental error. Group 5 shows a somewhat higher P^{32} content but this is probably not significantly different from Groups 1 and 2.

In a third series, 0.2 mg HS was injected 24 hours after hepatectomy and allowed to act for 10, 24, and 48 hours. Mitotic counts were taken but P^{32} was not followed. Results were as shown in Table III.

Although mitosis is lower in livers examined 24 hours after HS administration than in controls, yet it is higher in those removed 48 hours after HS treatment. The results, as in the previous experiment, suggest that the HS inhibition is temporary, and, upon elimination of HS from the liver, the rate of mitosis increases. Since HS has no effect on the P^{32} uptake of the tissue comparable to that on mitosis, the results suggest that the inhibition observed is not produced by way of the cellular phosphorylating mechanism.

The inhibition of mitosis by HS suggested analogy with similar effects of x-rays which also inhibit mitosis in the nuclear resting stage. A search was therefore made for chromosome aberrations comparable to those found after x-ray treatment. None were observed. In an earlier study, it was shown that treatment with x-rays which also inhibits mitosis in the resting stage produces little if any change in the P^{32} taken up by liver or tumor tissue; however, the distribution of P^{32} between nucleus and cytoplasm was very markedly altered.[4] Obviously, therefore, further work is needed before final conclusions are reached on the effect of HS on the cellular and nuclear metabolism of phosphorus.

Summary. 1. Within 3 hours after intravenous injection, 0.1-0.4 mg of βis (β-chloroethyl) sulfide per 50 g rat produces a marked reduction in the number of cells in metaphase and anaphase in regenerating rat liver. A dose of 0.2 mg inhibits mitosis for at least 24 hours. By 48 hours, this inhibition is released. If the mustard reaches the liver before mitosis is initiated the cells are prevented from entering into mitosis.

2. There is no significant change in P^{32} uptake by liver cells during the interval 3-23 hours after the mustard is injected.

3. Comparison is made with the effects of X-rays on mitosis and P^{32} uptake. No chromosome abnormalities were observed at the dosage levels used.

[4] Marshak, A., *J. Gen. Physiol.*, 1941, **25**, 275.

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Application of Gersh's Ferrocyanide Technique to the Study of Experimental Renal Disease.

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Gersh, using histochemical methods, demonstrated in the rabbit, dog, monkey, and other species[1,3] that sodium ferrocyanide was excreted solely by glomerular filtration without apparent tubular reabsorption or secretion;

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[1] Gersh, I., and Stieglitz, E. J., *Anat. Rec.*, 1933-34, **58**, 349.

[2] Van Slyke, D. D., Hiller, A., Miller, B. F., *Am. J. Physiol.*, 1935, **113**, 611.

[3] *Ibid.*, personal communication from Gersh to the above authors, of p. 613.

these findings have been confirmed by clearance methods.[2] The technic as originally described has been slightly modified and found to be of value in investigating renal function in experimental kidney lesions in dogs and rabbits.

Method. The animal is given intravenously 5 cc per kg of a 10% solution of sodium ferrocyanide [$Na_4Fe(CN)_6 \cdot 10H_2O$]. This solution is injected rapidly. After an interval of approximately 5 minutes, or as soon after the completion of the injection as ferrocyanide is detected in the bladder washings (it may be detected almost immediately after the initiation of the injection in a normal dog, or it may not appear at all, if there is severe renal damage), the animal is lightly anesthetized with intravenous sodium pentothal (15 mg per kg) and the left kidney exposed by a subcostal flank incision, but not manipulated in any way. A second intravenous injection of sodium ferrocyanide solution is given in the same dosage as the first and, simultaneously with its completion, the pedicle of the exposed kidney is clamped and cut and the kidney removed. Sections 1-2 mm thick are immediately cut and, supported on squares of copper screen, are dropped into a beaker of hexane which has been cooled to approximately −80°C by immersion in a bath of solid CO_2 in methyl-cellosolve. After a few minutes the frozen kidney slices are transferred to a dry flask chilled to −80°C. This is then connected to the manifold of a lyophilizing apparatus and the tissues are dehydrated *in vacuo* for 24 hours. The temperature of the flask containing the sections is maintained at the beginning of the dehydration at −25° to −35°C by a bath of equal weights of ice and chilled 95% ethyl alcohol in a Dewar flask and during the last 4 hours of this period the flask containing the kidney slices is allowed to come from approximately −10°C to room temperature. Melted paraffin is added to the warmed flask and the dehydrated tissues are infiltrated with paraffin by evacuation of the air in the flask; sections are cut at 25 micra and mounted without contact with water by gently spreading them on a warmed slide. The mounted sections are then exposed (dropping bottle technic) in succession to saturated solutions of ferric chloride in xylol and absolute alcohol and a 2% solution in 95% ethyl alcohol. Rinsing in 95% ethyl alcohol, dehydration in absolute ethyl alcohol, clearing in xylol and mounting in clarite then follows. A duplicate section may be counterstained by alcoholic eosin after having been run through the alcoholic ferric chloride solutions. In examining the sections that are not counterstained, the use of a substage lamp with a pink filter facilitates the detection of small amounts of the blue ferric ferrocyanide precipitate.

Results. A section prepared in this manner from a normally functioning kidney usually shows a granular blue deposit within Bowman's capsule and a blue-staining of the tuft and capsule itself. At times there may be no deposit, but the blue-staining of the capsule indicates that ferro-cyanide has been filtered. The purpose of the second injection of sodium ferrocyanide is to insure that there will be a deposit of ferric ferrocyanide within Bowman's space; often none can be found here in a kidney with normal function after only one injection, as recommended by Gersh. A blue deposit is detectable within the lumen of the proximal convoluted tubules, or adherent to the margin of their cells. (These tubules often may be somewhat distorted by artefact.) In the remainder of the tubular system the deposit in the lumen is heavier than in the proximal convoluted tubules.

Various experimentally produced renal disturbances have been studied by the use of this method. Acute hydronephrosis was produced by ligation of one of the ureters and the kidneys studied 24 hours later. In the hydronephrotic kidney, ferrocyanide precipitate was plentiful in the glomerular spaces and in most of the proximal convoluted tubules, scanty and only irregularly present in the loops of Henle and distal convoluted tubules and usually absent in the collecting tubules. In the control kidney the precipitate was found throughout the tubular system.

In a dog that had received 3.5 mg per kg of $HgCl_2$ 24 hours previously, the ferrocyanide deposit was plentiful in the glomerular spaces. The necrotic cells of the proximal convoluted tubules were stained diffusely blue but no

precipitate was found in the rest of the tubular system.

The method has also been used in the study of the renal injury produced by intravascular hemolysis and by the injection of solutions of hemoglobin. These results will be reported in detail subsequently.

Summary. Gersh's ferrocyanide histochemical renal function technic, slightly modified, has been found useful in studying experimentally produced renal lesions. In contrast to the finding of Gersh that such preparations fade rapidly, these have shown no change after 9 months.

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A Dialyzable Medium for Cultivation of Group A Hemolytic Streptococci.*

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In the course of work with the filtrates of streptococcal cultures a need was felt for a purified medium capable of supporting abundant growth of the organisms. Synthetic media of the present day did not appear to meet the requirements fully since no one of these media is uniformly capable of supporting heavy growth of all pathogenic strains of interest. Ultimately such defined media should be sufficiently developed to supplant the procedures described in this report.

Since it had previously been shown by Stock[1] that some toxin-producing streptococci are capable of growing on a medium containing only dialyzable constituents, a trial was made with heart muscle extract and commercial peptone purified by preliminary dialysis. It was found that the medium made with these purified constituents actually allowed more rapid and abundant growth in most cases than the standard Todd-Hewitt broth[2] of similar composition. The success of these efforts suggested that the medium might find a wider use than had originally been contemplated.

Preparation of the Purified Beef Heart Extract. Beef heart, after being stripped of fat and finely ground, is soaked overnight at 4°C in water,† 250 cc per pound of meat. Following this infusion the mixture is heated to 85°C for 30 minutes. This amount of heating appears to be just sufficient to coagulate muscle proteins and to express intracellular fluid, yet sufficiently mild to spare most of the heat-labile growth factors. A ten-fold reduction in the quantity of meat required is made possible by this minimal exposure to heat; with more violent extraction or with subsequent heat sterilization of the completed medium, a larger quantity of meat is needed

At the end of the 30-minute heating period, the extract is filtered through fluted filter paper (supported under the point of the cone with cheese-cloth) and the juice expressed with a wooden masher, then cooled by immersion of the receiving flask in running water. The pink, slightly turbid filtrate, now about 300 cc per pound of meat, is introduced into cellophane casings‡ each about one meter in length and suspended in the upper two-thirds of a suitable tall, narrow vessel against 2 vol-

* This investigation was carried out under a contract between the Rockefeller Institute for Medical Research and the Commission on Hemolytic Streptococcal Infections, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army.

1 Stock, A. H., *J. Immunol.*, 1939, **36**, 489; *J. Biol. Chem.*, 1942, **142**, 777.

2 Todd, E. W., and Hewitt, L. F., *J. Path. and Bact.*, 1932, **35**, 973.

† No difference in the rate or amount of growth was observed when New York City tap water was compared with distilled water.

‡ Visking Cellulose Sausage Casing, "NoJax" 27/32.