TABLE IV. Co^{60} Excretion following Administration of Graded Doses of Intrinsic Factor and Labeled Vitamin B_{12} to Patients with a Total Gastrectomy.

Subject	Dose: Vit. B_{12}, μg	Dose: Intrinsic factor	Co^{60} excreted, %	Vitamin B_{12} absorbed (calculated) μg
Mo	.5		100	0
	.5	2.5 mg conc.*	64	.18
	.5	5.0 *	28	.36
	5.0		91	.45
	5.0	200 †	65	1.74
	5.0	400 †	62	1.93
St	.5		95	.03
	.5	25.0 mg conc.†	74	.13
	.5	50.0 †	28	.36
	5.0		87	.65
	5.0	150 ml gastric juice	40	3.00
	5.0	300	38	3.10

* Supplied by Merck & Co., Inc.
† " " Upjohn Co.

ent experiments, the formation of a similar compound which is not readily absorbed might explain the inhibitory results and serve also as a basis for explaining the limitation of absorption observed when doses of the order of 5 to 10 μg of B_{12} alone were given. At this concentration of vitamin the cobalamin protein might be formed within the gastrointestinal tract.

Summary. The results show the extreme limitation of vit. B_{12} absorption from the gastrointestinal tract of individuals with normal gastric function. Within the dose range used, an upper level of absorption appears to have been reached which averages 1.6 μg vit. B_{12}. This level is not increased by giving the vitamin with a test meal or in conjunction with intrinsic factor sources. In patients with a total gastrectomy, a graded response to intrinsic factor is obtained using 0.5 μg vit. B_{12} but at a 5 μg level, increasing the amount of intrinsic factor does not increase B_{12} absorption beyond levels obtained in normal subjects.

1. Swendseid, M. E., Gasster, M., and Halsted, J. A., *Fed. Proc.*, 1954, v13, 321.
2. Heinle, R. W., Welch, A. D., Scharf, V., Meacham, G. C., and Prusoff, W. H., *Trans. Assn. Am. Phys.*, 1952, v65, 214.
3. Callender, S. T., Turnbull, A., and Wakisaka, G., *Brit. Med. J.*, 1954, v1, 10.
4. Swendseid, M. E., Halsted, J. A., and Libby, R. L., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 226.
5. Halsted, J. A., Gasster, M., and Drenick, E., *New Engl. J. Med.*, 1954, v251, 161.
6. Berk, L., Castle, W. B., Welch, A. D., Heinle, R. W., Anker, R., and Epstein, M., *ibid.*, 1948, v239, 911.
7. Glass, G. B. J., Boyd, L. J., and Stephanson, L., *Fed. Proc.*, 1954, v13, 54.
8. Rosenblum, C., Woodbury, D. T., Gilfillan, E. W., and Emerson, G. A., *ibid.*, 1954, v13, 475.
9. Wijmenga, H. G., Thompson, K. W., Stern, K. G., and O'Connell, D. J., *Biochim. Biophys. Acta.*, 1954, v13, 144.

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Regeneration of Tissue Nonprotein Sulfhydryl Compounds in Rats After Exposure to Cold and Restraint.* (21247)

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It has been previously reported(1-4) that cold and restraint lower the concentration of liver nonprotein sulfhydryl compounds. Since Waelsch and Rittenberg(5,6) and Block(7,8) reported a rapid turnover of liver glutathione, this study was done to determine if the regeneration of these sulfhydryl compounds would be equally fast after the concentration was lowered by exposure to cold and restraint. To observe the effect of caloric intake on the

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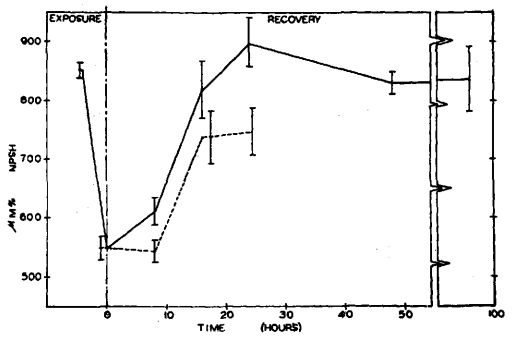

FIG. 1. Effect of fasting (hatched line) and nonfasting (solid line) on regeneration of liver nonprotein sulfhydryl compounds in rat after exposure to cold and restraint. Stand. error of mean indicated by vertical lines on the curves.

rate of regeneration, food was withheld from some of the animals.

Methods and materials. Eighty-five female Sprague-Dawley rats weighing between 175 and 200 g were treated as follows: 10 animals were used as controls. The remaining 75 animals were exposed to cold and restraint, and body temperature drop was regulated to 5° per hour so that at the termination of a 4-hour exposure the body temperatures were approximately 18°C. Ten animals were sacrificed at the termination of this exposure period. The other 65 animals were divided into 2 categories. Twenty-one of the rats were given no food and groups of 7 were sacrificed at 8, 16, and 24 hours respectively. Forty-four of the rats were given food *ad libitum* and groups of 7 were sacrificed at 8 and 16 hours, and groups of 10 were sacrificed at 24, 48, and 96 hours respectively. The animals were not restrained during the recovery period during which time they were placed in individual laboratory cages. All of the animals were stunned with a blow on the head, decapitated to avoid congestion of the organs with blood, and the tissues taken immediately and frozen in powdered dry ice. The total non-protein sulfhydryl concentration (NPSH) of the liver and kidney was determined using a modification of the method of Benesch and Benesch (9).

Results. As seen in Fig. 1, regeneration of the lowered liver NPSH was virtually completed by 16 hours in the nonfasting animals. At 8 hours there was little regeneration of the sulfhydryl compounds. In the fasting animals regeneration was somewhat slower in starting, and recovery of control liver levels was not complete in 24 hours.

As observed from Fig. 2, it appears that there was a small drop in the kidney NPSH as a result of exposure to cold and restraint. In both the fasting and nonfasting animals control levels were reestablished in less than 8 hours. In both of these categories there seemed to be an overcompensation so that there was an increase in kidney NPSH above the control values. In the nonfasting animals control values were reestablished again in 48 hours.

Discussion. The rate of regeneration of NPSH in the liver after exposure to cold and restraint was much less than the rate of loss during exposure to these stresses (Fig. 1). However, until normal body temperatures had been established (5 or 6 hours), maximum regeneration of sulfhydryl compounds would probably not be expected. It can be seen from Fig. 1 that there was no significant increase in the liver NPSH 8 hours after removal from the cold. Even at 16 hours recovery was not complete.

Since glutathione (GSH) comprises nearly all the NPSH in the liver (ergothionine, which comprises less than 10% of the NPSH, is not altered by cold and restraint(3)) it would be expected that the half life of NPSH would be approximately that of GSH. Waelsch and Rittenberg(5,6) suggested that the half life of liver GSH was 2 to 4 hours in normal ani-

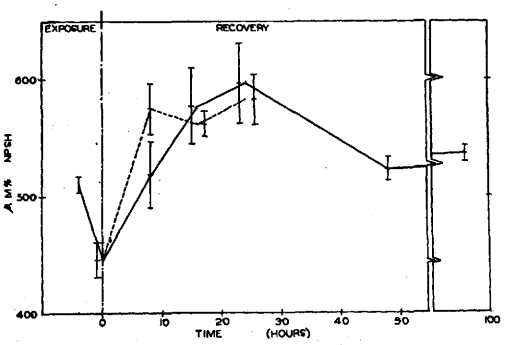

FIG. 2. Effect of fasting (hatched line) and nonfasting (solid line) on regeneration of kidney nonprotein sulfhydryl compounds in rat after exposure to cold and restraint. Stand. error of mean indicated by vertical lines on curves.

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.

1. Beck, L. V., and Linkenheimer, W. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 291.

2. Linkenheimer, W. H., and Beck, L. V., *Fed. Proc.*, 1953, v12, 88.

3. Bartlett, R. G., Jr., and Register, U. D., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 708.

4. Bartlett, R. G., Jr., *ibid.*, 1954, v86, 397.

5. Waelsch, H., and Rittenberg, D., *J. Biol. Chem.*, 1941, v139, 761.

6. Waelsch, H., *ibid.*, 1942, v144, 53.

7. Block, K., and Anker, H. S., *ibid.*, 1947, v169, 765.

8. Block, K., *ibid.*, 1949, v179, 1245.

9. Benesch, R. E., and Benesch, R., *Arch. Biochem.*, 1951, v30, 217.

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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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