years previous to the study, had both a higher glycoprotein (136 v. 125 mg/100 ml) and a higher serum protein (7.74 v. 7.16). A second monozygotic pair included one twin with a history of a cold one week previous to sampling (glycoprotein 133 mg/100 ml v. 122 for the other twin).

Summary and conclusions. The influence of genetic factors on the levels of total serum glycoprotein hexose, seromucoid hexose, and the bound hexose of electrophoretic fractions were investigated by comparing the differences found between monozygotic twin pairs as contrasted to the differences between dizygotic twin pairs. The differences between total serum glycoprotein hexose of the monozygotic twin pairs were significantly lower than those found for the dizygotic twins. The quantity of hexose bound to various electrophoretic fractions was found to be closer between monozygotic than between dizygotic twins. However, these differences were not significant statistically. It may be concluded that genetic factors play a minor role in establishment of normal levels of serum protein and glycoprotein.

1. Stary, Z., *Clinical Chem.,* 1957, v3, 557.
2. Winzler, R. J., *Methods of Biochemical Analysis,* 1955, v2, 279. Interscience Publishers, New York.
3. Lustig, B., Novak, J., *J. Mt. Sinai Hosp.,* 1947, v14, 534.
4. Shetlar, M. R., Foster, J. V., Kelly, K. H., Everett, M. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 507.
5. Shetlar, M. R., Kelly, K. H., Foster, J. V., Shetlar, C. L., Everett, M. R., *Am. J. Ob. and Gyn.,* 1950, v59, 1140.
6. Newman, H. H., *Bulletin Biology* 19, v55, 283.
7. Shetlar, M. R., Cahill, C., Stidworthy, G., Shetlar, C. L., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 44.
8. Shetlar, M. R., Foster, J. V., Everett, M. R., *ibid.,* 1948, v67, 125.
9. Weimer, H. E., Redlich-Moshin, J., *Am. Rev. Tuberc.,* 1952, v68, 594.

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Studies on Role of Liver in Nitrogen Mustard Detoxication.* (24272)

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Skipper *et al.,* who investigated the distribution of methyl-C^{14}-bis (beta-chloroethyl) amine, (HN2), in mice observed that appreciable amounts of radiocarbon were excreted in the form of carbon dioxide(1). In our laboratory similar results were obtained in rats. It was shown that only small amounts of the C^{14}-methyl group of HN2 were transferred by transmethylation mechanisms(2). Subsequent studies showed that in the microsomal fraction of rabbit and rat liver homogenates there existed an enzyme system which could readily demethylate and oxidize the labile methyl group of HN2. It also was demonstrated that such an *in vitro* preparation removed the N-ethyl group of HN2, although at a slower rate than the methyl group(3). Thus, there was a possibility that the liver, through demethylation and de-ethylation, was able to metabolize sufficient amounts of the injected drug in a short time to justify the assumption that the observed de-alkylations represented a detoxication mechanism.

This investigation was undertaken with the aim of determining how much radioisotope was excreted by the liver *via* the lymphatic and biliary systems after intravenous administration, and if these systems still contained significant amounts of cytotoxic materials. Additional studies were carried out in order to determine if preferential routing of the HN2 through the liver influenced the toxicity of the drug.

Methods. The methyl-C^{14} bis (beta-chloro-

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ethyl) amine HCl was obtained from the U.S. Atomic Energy Commission and had a specific activity of 15.2 μc per mg. Radioactivity was assayed in a continuous gas flow proportional counter on infinitely thin plated samples. All animals used in this study were adult Osborn-Mendel rats. Collection of lymph or bile was accomplished under sterile conditions through cannulae in the thoracic duct and/or the common bile duct. The animals were placed in restraining cages and the fluids collected from PE 10 polyethylene tubing while the animals were fed and watered *ad libitum*. Non-labeled nitrogen mustard ("mustargen-Merck") was injected under ether anesthesia at concentrations of 1.0 mg/ml through the femoral vein or, following laparotomy, through the vena cava or the portal vein of the rat.

Toxicity of bile or lymph obtained from the rats was estimated by tissue culture assay. Fragments of embryonic C3H mouse hearts were grown in roller tubes. The nutrient (Gey's) was withdrawn after growth was established and exchanged for nutrient-bile or nutrient-lymph mixtures. Toxicity of bile or lymph was determined on fragments cultured for 48 hours. The tissue cultures were exposed to lymph or bile for 6 hours. Following restitution of the normal nutrient the individual fragments were graded 18 hours later on the basis of cellular rounding, granulation, and the extent of disintegration and compared with control tubes. At least 20 tissue culture fragments were employed for each determination of cytotoxicity.

Results. About 30% of the intravenously administered radioactivity from C^{14}-methyl labeled HN2 was excreted in 24 hours through the common bile duct. During the same period 15% of the radioactivity was collected in thoracic duct lymph.

Comparison of specific activity of bile, lymph and blood indicated that bile contained 3 to 4 times the radioactivity of the lymph and from 25 to 35 times that of blood at comparable intervals after injection of the radioactive drug. Assay of bile obtained at varying intervals after injection of HN2 into the rat showed that this bile did not contain sufficient cytotoxic material (or unchanged drug) to account for the radioactivity observed in these samples (Table I). Subsequent chromatographic studies indicated that there was little or no unchanged drug present in the bile. Exposure of tissue culture fragments to diluted samples of thoracic duct lymph showed that lymph contained some residual cytotoxic material (Table I). Calculations based on amount of radioactivity in samples withdrawn 2 and 4 hours after injection indicated that one-tenth of the radioactivity may have been present in the form of cytotoxic material or as unchanged HN2. Lymph and bile obtained from untreated rats failed to protect tissue culture fragments against the deleterious effects of added HN2. To determine if passage of HN2 through the liver prior to reaching the systemic circulation had an effect on overall toxicity of HN2, the drug was injected at an LD_{50} level *via* the portal vein. Control animals received the HN2 by injection into the vena cava or femoral vein. There was no significant difference between survival rates of the portal vein injected and femoral vein injected rats (Table II). The reason for the

TABLE I. Effect of Bile and Lymph from HN2 Treated Rats on Embryonic Mouse Heart Tissue Cultures.

Substance tested	Conc. of HN2 in mg/ml	% of clonal fibroblasts showing: Rounding	Granulation	% of clones showing lysis and disintegration
Bile	5×10^{-4} *	20-25	20-25	10-20
"	1×10^{-3} *	75-95	75-95	75-95
"	1×10^{-2} †	0- 5	10-20	0- 5
"	4×10^{-2} †	0- 5	25-50	0- 5
Lymph	1×10^{-3} *	75-95	75-95	75-95
"	1×10^{-3} †	0- 5	50-75	0- 5
"	4×10^{-3} †	50-75	75-95	50-75

* Known amounts of HN2 added as Mustargen.
† Amounts of HN2 calculated on basis of C^{14} content.

TABLE II. Effect of Route of Administration of an LD_{50} Dose of HN2 on Mortality Rate of Adult Rats.

Route of admin.	No. rats	No. rats died within 10 days	No. rats survived
Femoral vein	20	12	8
Vena cava	17	14	3
Portal vein	20	13	7

slightly higher mortality rate in vena cava injected animals was not evident.

Summary and conclusion. It was concluded that a major portion of intravenously injected HN2 passes through the liver of the animal. There, metabolic alterations, possibly other than simple de-alkylations, take place which lead to the biliary or lymphatic excretion of compounds the toxicity of which is greatly attenuated. It was demonstrated, however, that a single passage of the drug through the liver does not suffice to influence mortality rate due to an LD_{50} dose significantly. Detoxication of HN2 or its immediate metabolites in the liver, does not proceed at a sufficiently rapid rate to change the clinical course of mustard intoxication. This substantiates our previous observations which indicated that the effects of the drug on the organism are initiated quite rapidly following its administration.

1. Skipper, H. E., Bennett, L. L., Langham, W. H., *Cancer,* 1951, v4, 1025.

2. Trams, E. G., Klopp, C. T., *Cancer Research,* 1955, v15, 617.

3. Smith, P. K., Nadkarni, M. V., Trams, E. G., Davison, C., *Ann. N. Y. Acad. Sci.,* 1958, v68, 834.

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17-Ketosteroids in Gastric Juice.* (24273)

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The finding of 17-ketosteroids (17-KS) in gastric juice(1) warrants a more extended investigation. The present report comprises analyses of 28 samples obtained from 16 patients who were accepted at random and without consideration of age, sex or diagnosis.

Methods. Samples of gastric juice were obtained in 2 ways: 1) aspiration of the stomach one hour after instillation of 200 cc of 10% glucose in water or, 2) aspiration by Levine tube attached to the Wangensteen suction apparatus. Those obtained by simple aspiration were taken in the morning from an otherwise fasted stomach; material removed from the suction system was taken at undetermined times. After determination of the pH and adjustment to 1.0 or slightly less, the samples were boiled for 10 minutes under a reflux condenser, cooled and extracted with ether. Separation with the Girard T reagent was practiced and the extract was again evaporated to dryness. The resultant dry material was dissolved in 5 cc of freshly distilled ethyl alcohol. After development of color with dinitrobenzene and KOH, the aliquots were read in the Coleman spectrophotometer. A standard curve was developed with each lot of unknowns.

Results. The results are detailed in the accompanying table. Values have been calculated on the basis of milligrams per liter of gastric juice.

Discussion. Comparison of the pH values with those of the 17-KS fails to suggest a direct correlation. Of greater interest is the magnitude of some of the quoted values in comparison with the negligible concentrations of 17-KS found in blood plasma. In this connection, Samuels *et al.*(2) reported that there are no measurable amounts of 17-KS in peripheral human blood. Other investigators(3, 4) using modified procedures, have carried out successful analyses of plasma for dehydroepiandrosterone (DHA) and androsterone. On the basis of these individualized methods, Gardner quotes values for normal males of 40-130 μg/100 ml, with somewhat lower figures for females. Recalling that DHA could hardly be expected to be present in association with the pH commonly found in gastric juice,

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