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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TABLE I. 17-Ketosteroids in Gastric Juice.

Case	Sex	Date (1958)	Clinical notes	pH	& color	17-Keto. (mg/l)
1	♀	5/23	Postoperative cholecystectomy and hemicolectomy	4.5	Green	*5.0
		24		1.3	"	4.8
2	♀	23	Adrenalectomy; during surgery. Hydrocortisone IV	6.3	"	9.2
		24	Postoperative—24 hr	3.4	"	.4
3	♀	6/10	Diverticulitis with obstruction	2.1	"	2.6
		10		1.7	"	3.2
		13		1.4	"	trace
4	♀	24	Small bowel obstruction, prior to surgery; on hydrocortisone IV.	1.7	"	8.2
5	♂	15	Postoperative; partial gastrectomy	6.7	Buff	0
		15		6.7	"	0
		16		6.8	Orange	trace
6	♀	15	Colectomy. Hydrocortisone, 100 mg IV.	1.7	Yellow	4.6
7	♂	18	Partial gastrectomy	6.8	Buff	2.0
8	♂	5/27	Gastric ulcer	1.7	Colorless	†5.0
9	♂	27	Cholecystitis	2.0	"	4.2
10	♂	29	No gastro-intestinal disease	2.5	"	2.5
11	♂	29	Bleeding peptic ulcer	1.4	"	6.0
12	♂	29	Pyloric ulcer	3.0	"	4.6
13	♂	29	Cirrhosis of liver	2.3	"	2.9
14	♂	6/ 6	Duodenal ulcer	1.7	"	4.0
15	♂	6	Rheumatic heart	1.8	"	1.4
16	♀	3/22	Chronic nephritis with uremia			1.0
		24	*Idem*			2.8
		28	"			2.6
		28	"			2.6
		28	"			2.0
		4/12	"			0
		12	"			0

* Aspiration by constant suction. † Simple aspiration.

nor would it have survived the acid hydrolysis to which our samples were subjected, it is probably safe to assume that the values found in gastric juice are far in excess of what might be present in the plasma and are comparable to those found in urine.

On the other hand, intimations of correlation can be found, even in these few preliminary analyses, with intravenously administered hydrocortisone. Most strikingly suggestive is the second case, in which the first specimen was taken during surgical removal of the adrenals, while 100 mg of hydrocortisone were being administered by intravenous drip. The second sample was withdrawn on the following day, at which time the replacement therapy was given in smaller doses, by the intramuscular route, and 17-KS concentration had dropped from 9.2 mg per liter to 0.4.

It will be noted that the instances in which 17-KS were absent entirely or present only in trace were 2, a male patient who had undergone partial gastrectomy, and a female who was in the pre-terminal phase of chronic glomerulonephritis with high-grade uremia. Further studies are required for even a tentative interpretation of these findings.

Conclusions. Ether-soluble material giving a positive Zimmerman reaction is present in gastric juice in varying concentrations. Evidence is lacking of a direct correlation between acidity of the juice and concentration of the steroids. Concentration of 17-KS in gastric juice is shown to increase markedly during intravenous administration of hydrocortisone; hence it appears to reflect to some extent the blood level of hydrocortisone. The inference is justified that the gastric mucosa is the site of

active steroid metabolism.

1. Farnsworth, E. B., *Quart. Bull. Northwestern U. Med. School,* Fall, 1958, v32.
2. West, C. D., Tyler, F. H., Brown, H., Samuels, L. T., *J. Clin. Endocrinol.,* 1951, v11, 897.
3. Gardner, L. I., *J. Clin. Endocrinol. and Metab.,* 1953, v13, 941.
4. Migeon, C. J., Plager, J. E., *J. Clin. Endocrinol.,* 1955, v15, 702.

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Assay of "Hemopoietine" in Starved Animals; Properties of Urinary Hemopoietine. (24274)

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The effects of plasma from anemic rabbits on plasma iron turnover of normal rabbits have been described(1). A clear increase in plasma iron turnover was produced by native and boiled plasma from phenylhydrazine treated rabbits; bled rabbits' plasma produced a slighter effect and only after dialysis. Although rich in Hemopoietine, plasma from phenylhydrazine treated animals is not an economic source for abundant material necessary for chemical fractionation studies. In view therefore of our previous findings that "Hemopoietine" appears in urine(2,3), it was decided to use urine from anemic animals, as a source of "Hemopoietine" for chemical studies. Before doing this it was necessary, however, to work out a satisfactory method for Hemopoietine concentration, and also have available an adequate test for assaying the various fractions quantitatively. Use of plasma iron turnover studies in normal rabbits as a routine assay is impractical, because of large amounts of material required. Preliminary experiments with normal rats, measuring Fe^{59} uptake in erythrocytes, indicated that this was not a satisfactory bio-assay animal either. It was with great interest therefore that we read of the experiments which demonstrated that animals with depressed erythropoiesis, presumably a consequence of low endogenous hemopoietine titers, were very sensitive to extracts from plasma of anemic animals(4,5).

This communication presents results of experiments in which starved rats with depressed erythropoiesis(5), were used as assay animals to develop a method for Hemopoietine concentration, and to describe the application of this method to the study of some properties of urinary Hemopoietine.

Method donor animals. Rabbits of both sexes, average weight 2 kg, were used. Treatment of these animals has been described(1). For urine collection the rabbits were maintained in metabolic cages, and urine collected in sterile glass flasks kept in an ice-salt mixture. The urine obtained was centrifuged and then kept frozen until further treatment.

A. *Receptor rabbits.* To test the effect of Hemopoietine on Hb synthesis in starved animals, extract of boiled plasma from phenylhydrazine treated rabbits prepared according to Borsook(6), were injected intravenously (10 ml/kg), twice a day for 2 days, in fasted male rabbits (2 kg). Animals were fasted 2 days before and during injection period. Fe^{59} was injected on morning of 5th day of fasting, and plasma iron turnover and Fe^{59} incorporation into red cells studied by methods previously described(1). The results of this experiment are summarized in Table I.

B. *Receptor albino rats.* In preliminary tests for adequate method of concentration for Hemopoietine, starved male albino rats, of weights indicated in Table II, were used. These rats were fasted 2 days previous to Hemopoietine injections and for 3 days thereafter. Hemopoietine preparations were injected intravenously (tail vein) twice a day on third and fourth day of fasting. Fe^{59}, 0.2