Table 1. Blood-type frequencies.

Typed	Total cases	Blood groups							
		O		A		B		AB	
		No.	%	No.	%	No.	%	No.	%
Gastric carcinoma patients	879	370	42.09	403	45.85	81	9.22	25	2.84
Peptic ulcer patients	1770	946	53.45	655	37.01	128	7.23	41	2.31
Controls	6313	2892	45.81	2625	41.58	570	9.03	226	3.58

patients seen and treated at the university and five other Iowa hospitals during the past 20 years (*5*). Unselected, consecutive cases of gastric carcinoma and peptic ulceration are the clinical material upon which this preliminary report is based. A histologic diagnosis, resulting from study of surgical or post-mortem tissue sections, was required for inclusion of carcinoma cases. Duodenal and gastric ulcer cases were included when (i) an iron-clad clinical diagnosis existed that was based on clinical, x-ray and/or gastroscopic findings, or (ii) a gross or microscopic diagnosis was established at the time of surgery or post-mortem examination. In order to insure that these criteria would be rigidly adhered to, all case records were reviewed by one or another of us. The extracted data were transferred to IBM punch-cards and sorted by machine methods. In order to test the significance of our results, they were subjected to statistical analysis by means of the chi-square and difference-between-percentages methods (*6*).

It was of the greatest importance to establish reliable values for blood-type frequencies among healthy individuals who were to be used as controls for comparison with the blood-type frequencies observed in the patient groups. The blood types of consecutive blood donors contributing to two hospital blood banks where most patient typings were done were recorded. Before giving blood, these individuals were screened to eliminate those with disorders contraindicating their use as donors. For the most part, they were relatives or acquaintances of the patients. Therefore, most of them came from the same population groups as the patients and had ethnological backgrounds similar to those of the patients.

The blood-type frequencies and the percentages these represent of the total control and patient groups are recorded in Table 1.

When blood-type frequencies in the control and patient groups are compared, it becomes clear that two significant associations exist. Members of blood type A are either more susceptible to, or have less resistance against, carcinoma of the stomach than members of the three other blood types. The significance of this relationship is at the 2-percent level. Members of blood type O, on the other hand, appear to have even greater vulnerability to, or less resistance against, peptic ulceration. The degree of significance here is at the significant level of 0.1 percent.

It is evident that the possession of blood type A is related to natural selection, in that such individuals have a greater probability of developing carcinoma of the stomach and consequently are less favored than members of the other blood types. Conversely, members with these other types are thus favored, except for the selection exercised on group O members by the increased likelihood that they will develop peptic ulceration.

The association of blood types, the genetics of which have been extensively studied and are well understood, to carcinoma of the stomach fits well with the heretofore suspected familial predisposition to this and other malignancies. A similar genetic factor, acting to cause peptic ulceration, is suggested by the demonstrated predisposition of type O individuals.

The hypotheses that may be advanced to explain these findings seem to fall into one of two general categories—that is, local factors acting in or on the stomach or general ones acting to control the individual's response to the carcinogen or ulcerogen. In the first instance, known mucopolysaccharide blood-group substances may act to increase tissue vulnerability to, or diminish resistance against, carcinogens and ulcerogens. In view of the large number of such substances that have already been isolated, resulting in the definition of the many blood groups, it seems likely that still other similar antigens remain to be identified that could well be responsible for these effects. Local tissue factors and enzyme activities that are unrelated to the red blood cell and its antigens remain less attractive possibilities. Perhaps more appealing, although little evidence thus far has been presented in its support, is the concept of fundamental, individual biochemical and/or physiological differences. Blood types, susceptibility to bacterial organisms, and predispositions to carcinogens and ulcerogens would reflect these fundamental differences in individuals, representing differences in response to similar environmental factors. Evidence that similar relationships do not exist for certain other malignancies and blood types as for carcinoma of the stomach does not rule out this explanation.

A relationship between natural selection and the ABO blood group is indicated by the results of this study through (i) a significant increase of type A among patients with carcinoma of the stomach, and (ii) a significant increase of type O among patients with peptic ulceration.

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5. Space does not permit acknowledgment of the individuals and hospitals participating in the collection of the data, which are to be included in more detailed reports that are now in preparation.
6. The statistical analysis was made by L. A. Knowler, department of mathematics and astronomy, State University of Iowa, Iowa City.

25 November 1955

Salivary Amylase in the Rat

In a review of the literature concerning amylase in the body fluids and tissues of the rat, no good quantitative data could be found on salivary amylase levels. Several sources (*1*, *2*) agreed that the rat does have amylase in its saliva but gave little other information. Quantitative data, of interest in this laboratory in connection with a larger study (*3*) on tissue amylases, have now been obtained and are reported here.

Fifteen Sprague-Dawley rats, fed on the usual laboratory diet, were used in this study. With the rats under ether anesthesia, flow of saliva was stimulated by intraperitoneal injection of approximately 1 mg/kg of pilocarpine (*4*). Saliva was collected by gentle suction on a micropipette leading to a small trap. By this means, 0.3 to 0.5 ml of saliva could be collected in 10 to 15 minutes. After saliva had been obtained, the rats were sacrificed, and blood was collected for serum amylase determinations.

Both salivary and serum amylase determinations were carried out by the method of Van Loon (*5*) (Van Loon's amylase units are numerically equal to Somogyi amylase units), using 1/100 dilutions of serum and 1/20,000 dilutions of saliva instead of the usual 1/10 dilution that is recommended for human serum. In an earlier study in this laboratory, human salivary amylase levels were found to be 50,000 to 200,000 units/100 ml, and 1/5000 dilutions of saliva were appropriate in the analyses. Incidentally, no diastaticlike action of pilocarpine (*6*) on starch or other interference with salivary amylase determination was noted.

The data in Table 1 show that there are wide variations in rat salivary amylase levels, but it is apparent that the average (626,000) is still several times that in human saliva. Thus, contrary to previous statements (*2*), man does not have "the highest ptyalin concentration of all animals."

These data on rats were of interest in view of the report (*7*) that in depancreatized rats, serum amylase levels and urinary amylase excretion remain essentially the same as in normal rats. Thus, some organ or organs other than the pancreas must be a source of rat serum amylase. The salivary glands might conceivably be such a source. However, comparison of salivary and serum amylase levels in rats showed little correlation. Also, in a rat that was sacrificed in this laboratory on the seventh day after surgical removal of its salivary glands, the serum amylase fell only slightly. The serum amylase preoperatively was 2500; 7 days postoperatively, it was still 1800. Since, with no saliva, the rat experienced some difficulty in eating, the fall in serum amylase level could be explained on a purely nutritional basis. Saline washings of this rat's mouth showed no trace of amylase activity.

Table 1. Salivary and serum amylase in rats.

Amylase (Van Loon units/100 ml)		Salivary/serum
Salivary	Serum	
1,340,000	4280	312
1,300,000	4810	270
1,180,000	4070	290
1,000,000	4070	245
750,000	4150	181
670,000	4230	158
630,000	3660	172
617,000	4400	140
510,000	3310	154
361,000	*	
307,000	3740	82
247,000	3660	67
205,000	4060	50
181,000	*	
91,000	3740	24

* Samples lost.

As one might have predicted, the amylase in rat saliva is apparently an α-amylase. Its action on soluble starch produced the progressive changes to the amylo-, erythro- and achrodextrin stages (as followed by the iodine reaction) that are typical of the action of α-amylase but not that of β-amylase.

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19 October 1955

Synthesis of 5-Hydroxypipecolic Acid and Separation of Its Diastereoisomers

The metabolic and structural relationships between ornithine and proline (*1*), hydroxyornithine and hydroxyproline (*2*), and lysine and pipecolic acid (*3*) prompted us to convert δ-hydroxy-L-lysine to 5-hydroxypipecolic acid, the homolog of hydroxyproline. This amino acid has recently been isolated from *Rhapis flabelliformis* (*4*) and has been obtained as an intermediate in the synthesis of baikiain (*5*).

δ-Hydroxy-L-lysine containing 18 percent *allo*hydroxy-D-lysine (*6*) was treated with 1.5 equivalents of nitrosyl chloride in 6*N* hydrochloric acid and kept at 60°C for 25 minutes. The reaction mixture was assayed for total nitrogen (Kjeldahl) and for α-amino nitrogen by evolution of carbon dioxide after oxidation with chloramine-T (*7*). It was found that about one-half of the total nitrogen was present as α-amino nitrogen. Assuming little or no formation of the α,ε-dichlorocaproic acid, it follows that approximately equal amounts of the α-chloro and ε-chloro acids were formed. The mixture was cyclized by bringing the aqueous solution to a *p*H of 11 with barium hydroxide and warming on the steam bath for 10 minutes.

The products of this reaction were studied by ion-exchange chromatography in an attempt to separate the diastereoisomers. The methods were patterned after those of Moore and Stein (*8*). A 150- by 0.9-cm column of Dowex 50-X8, 200 to 400 mesh (rescreened, wet, through 200 mesh), operated at room temperature and 4 ml/hr, was employed. Elution was started from a mixing chamber containing 130 ml of 0.3*M* citrate buffer (0.2*N* in sodium citrate) at *p*H 3.00. The *p*H was gradually increased by adding 0.2*N* sodium citrate to the buffer at a rate equal to one-half the elution rate. Buffer and citrate solution contained 1 percent of a detergent solution (*8*). One-milliliter fractions were collected. 5-Hydroxypipecolic acid was determined (*9*) by adding 1 ml of 2-percent ninhydrin in glacial acetic acid and placing the tubes in a boiling water bath for 15 minutes. After the mixture had been diluted with 5 ml of acetone-water (50/50 by volume), the concentration was determined from the absorbancy at 353 mμ.

Figure 1 shows a portion of the effluent curve that was obtained by chromatography of the reaction mixture from the cyclization of hydroxylysine. By admixture of an authentic sample of 5-hydroxy-L-pipecolic acid obtained from dates (*9*) or material kindly provided by A. I. Virtanen (*4*), the faster moving peak was identified as the natural isomer. The other peak was presumably the *allo* form since it showed an absorption spectrum after reaction with ninhydrin in glacial acetic acid identical with that obtained with the natural isomer. This was the same order of appearance as that observed for the diastereoisomers of hydroxyproline (*10*) on an ion exchange column, and it may be taken as a suggestion of the *trans* arrangement for the functional groups of 5-hydroxy-L-pipecolic acid. The yield from the cyclization reaction was only 2 to 5 percent.

Paper chromatography was also used to characterize the products of the cycli-

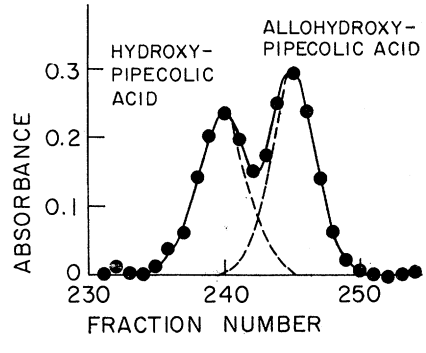

Fig. 1. Ion-exchange chromatography of the products of cyclization of δ-hydroxylysine showing the separation of the diastereoisomers of 5-hydroxypipecolic acid.