Strong,[10] biotin by the method of Shull, Hutchings and Peterson,[11] as modified by Shull and Peterson,[12] and folic acid as measured by both *L. casei*[13] and *S. faecalis*.[14] The results are given in Table 1.

It is evident that the four purified caseins carry significantly smaller quantities of all the vitamins studied than the crude casein. The actual amount varies from 1/10 to 1/2 of that present in the unpurified casein. The vitamin content of the four purified caseins is approximately the same except in the case of acid-washed casein, which showed a lower content of riboflavin. The purified caseins used in this study therefore can not be considered vitamin-free. The amount present may not affect the experimental results in most cases, but the amount supplied must be taken into account when vitamin requirements are calculated. The data presented in Table 1 are merely results for specific samples of casein and can not be used for routine calculations since each batch of casein may vary considerably.

While only certain known vitamins have been determined, it is probable that other unknown compounds stimulating the growth of experimental animals may be carried by purified casein. Although the vitamin content of alcohol extracted casein tended to be somewhat higher than that of the acid washed, studies with guinea pigs,[15] dogs and rats receiving sulfa drugs have indicated that alcohol-extracted casein may contain less of certain unknown nutritional factors.

The problem of obtaining a suitable protein source completely free of all growth-stimulating substances other than the essential amino acids has not been solved. The substitution of synthetic amino acids for the natural protein in experimental diets may be one means of solving this problem, but this can not be done without studying the effect of this change on the requirement of other factors.

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## THE RELATION OF ENDOCRINE GLANDS TO THE GASTRIC SECRETORY DEPRESSANT IN URINE (UROGASTRONE)[1, 2, 3]

THE purpose of this preliminary communication is to present results of experiments on the origin of urogastrone, a gastric secretory depressant in urine, and its relationship to certain of the endocrine glands.

The work to be reported here is part of a systematic investigation of the effect of certain endocrine glands on the production of urogastrone. Three of these endocrine glands, namely, thyroids, ovaries and pituitaries, have been removed to date from different series of dogs and collections of urine made from these animals.

Urine was collected from each of the following series of female dogs: six normal, six oöphorectomized, six thyroidectomized plus oöphorectomized, and separately from two dogs that were hypophysectomized. The transbuccal method for removing the hypophysis was employed on the latter two. Autopsies of these two dogs revealed that the hypophyses were removed. Serial sections of the hypothalamic regions of each brain were also examined and found to be normal.[4]

Urogastrone was prepared by the procedure used in Ivy's laboratory,[5] a modification of the Katzman-Doisy method originally employed by Sandweiss, Saltzstein and Farbman[6] in obtaining their urine extracts of pregnant and of normal women.

The five preparations of urogastrone made from these four series of animals were each tested for their effect on gastric secretion stimulated by histamine in both Heidenhain pouch and gastric fistula dogs. The dogs were fasted at least 24 hours before each experiment.

Between 25 and 53 gastric secretory (double hista-

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[3] The valuable surgical assistance of Dr. J. Thorstad and others of Harper Hospital during the early phases of this study is gratefully acknowledged.

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mine) studies were conducted in the usual manner with each of the five extracts, a total of over 200 experiments. Each experiment consisted of two phases: in the first phase, urogastrone was administered intravenously, followed by the subcutaneous injection of histamine. The gastric juice was then collected every ten minutes for a period of seventy minutes and titrated for free and total HCl. Three hours later, in the second phase of the experiment, histamine only was administered and the gastric juice was collected and titrated as previously. In a number of studies these two phases were reversed.

In accordance with previous reports the urogastrone prepared from our normal dogs inhibited gastric secretion in the majority of experiments after histamine injection (inhibition in 63 per cent., augmentation in 18.5 per cent., no change in 18.5 per cent. of the studies). The acidity was also reduced after urogastrone injection, but it did not always correspond exactly to the diminished flow of gastric juice.

Urogastrone prepared from the thyroidectomized plus oöphorectomized dogs inhibited gastric secretion in approximately the same per cent. of the experiments as the urogastrone prepared from our normal dogs referred to above. The extract prepared from the oöphorectomized dogs apparently contained less urogastrone.

The extracts prepared from the two hypophysectomized animals inhibited gastric secretion in only 5 out of 52 studies (9.5 per cent.). What is more important, however, is the fact that in 32 out of 52 studies (61.5 per cent.), there was noted a significant increase in the quantity of gastric juice. The augmentation sometimes reached nearly 200 per cent.

Our results were analyzed statistically by two methods.[7] In the first, our data (*i.e.*, the number of times the volume output of secretion was increased or decreased by the injection of the extract of the urine of normal and of hypophysectomized dogs) were placed in the "four-fold table" and the Chi square was calculated. A value of 21 was obtained, thus indicating that the difference in the effect of the two extracts could occur by chance in less than 1 to 10,000 such cases. In the second, the standard deviation of the means of the HCl output in milliequivalents and the standard error of the difference of the means were computed. This gave a critical ratio of 2.11 which implies that the difference in the milliequivalents of HCl output could occur by chance in less than five times out of 100 similar experiments.

Thus, urogastrone, which is now well recognized as a gastric secretory depressant in normal urine, is also present when both the thyroids and the ovaries are removed. However, if the hypophysis is removed, the depressant is diminished, if at all present, and in a majority of experiments there was an actual increase in gastric secretion following administration of the extract prepared from these animals.

## COMMENTS

Clinical studies and impressions appear to indicate that the pituitary-thyroid-gonad mechanism plays a role in human peptic ulcer.[6,8] It is well known that peptic ulcer is comparatively rare in women, but when it does occur, it is ameliorated by pregnancy and aggravated by the menopause.[8] Also, women with ulcer show a high incidence of endocrinopathies.[8] While ulcer in the adult is approximately five to ten times as frequent in men as it is in women, in children before puberty, though rare, it is equal in both sexes. Of interest also, is the fact that extracts prepared from urine of both pregnant and normal women have a greater prophylactic, therapeutic and "immunizing" effect on the experimental Mann-Williamson ulcer in dogs,[6,8,9] than extracts of urine from normal men. It is therefore probable that in the female the monthly reproductive cycle and the increased glandular function during active sex life and pregnancies (*i.e.*, the chemical interplay between the pituitary and the ovaries throughout the active menstrual life of the female), augment the production of ulcer "protective factors" and thus serve to prevent or benefit "peptic" ulcer. The absence of these in the male could result in the lesser protection against ulcer. Abnormalities in the glandular function in both the male and the female may affect the protective factors and be responsible for ulcer formation.

Our preliminary data are indicative of a relationship between the pituitary gland and gastric secretion. When the gland is removed, the dog's urine contains very little, if any, of the gastric secretory depressant. In addition, when its urine extract is administered to Heidenhain pouch or fistula dogs an increase in the quantity of gastric juice is noted. This may suggest that with a disturbance in the pituitary gland, factors come into greater play that augment the action of gastric secretory stimulants, and contribute to ulcer formation.

Further work is now in progress on the relationship of the pituitary gland to "peptic" ulcer.

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