However, in view of the extremely large variations in individual swimming times at these water temperatures it would seem that the use of lower water temperatures and the final colonic temperature would provide a more precise method of evaluating stress relationships.

The differences in swimming time at 37°C between the wild and albino mice, were large enough to require some explanation. Scheer (10) had suggested that a restricted caloric intake would result in a shorter swim by causing a lessening of physical capacity. Recent studies(4,5) have indicated the changes in diet would alter the time to exhaustion for swimming animals. The adequacy of the diet of the wild animals could not be determined and whether or not this factor was involved in the results cannot be predicted. Further studies are being planned.

Summary. The suggestions in the literature that there are differences in response between strains of domestic and wild animals of the same species, based on supposed differences in psychological and physiological behavior, have not been borne out by the present study. The wild and albino mice utilized in the current study showed no significant differences in swimming time to exhaustion, or colonic temperature at exhaustion. These observations were in direct opposition to those reported comparing wild and domestic rats. There were individual variations in swimming times at all water temperatures despite the utilization of a precise end point for exhaustion. A valuable criterion for exhaustion appeared to be the final colonic temperature as suggested by the relationship between swimming times, water temperature (at least between 20 and 37°C) and colonic temperature. Other factors markedly shorten the duration of swimming at bath temperatures of 15 and 42°C. Group swimming resulted in a 50% decrease in swim time due to factors other than those which induce exhaustion in individually swimming animals. In view of the large individual variations in swimming times, evaluation of various psychological and physiological stresses must utilize the most reliable methods of pinpointing exhaustion and not depend upon such subjective measures as disorientation of the test animal.

1. Baker, M. A., Horvath, S. M., Am. J. Physiol., 1964, v207, 1073.

2. Barbour, H. G., McKay, E. A., ibid., 1943, v140, 9.

3. Britton, S. W., Quart. J. Exp. Physiol., 1930, v13, 55.

4. Ershoff, B. H., Proc. Soc. Exp. Biol. and Med., 1951, v77, 488.

5. ———, J. Nutr., 1954, v53, 439.

6. Griffiths, W. J., Psychol. Rep., 1960, v6, 39.

7. Jacob, J., Michaud, G., Arch. Int. Pharmacodyn., 1961, v133½, 101.

8. Le Blanc, J. A., Proc. Soc. Exp. Biol. and Med., 1958, v98, 648.

9. Richter, C. P., Psychsom. Med., 1957, v19, 191.

10. Scheer, B. T., Dorst, S., Codie, J. F., Soule, D. F., Am. J. Physiol., 1947, v149, 194.

11. Spealman, C. R., ibid., 1942, v146, 262.

12. Tan, E. M., Hanson, M. E., Richter, C. P., Fed. Proc., 1954, v13, 150.

13. Wilber, C. G., Am. J. Physiol., 1959, v190, 457.

14. Wilber, C. G., Hunn, J. B., J. Appl. Physiol., 1960, v15, 704.

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Decomposition of 3-Hydroxyanthranilic Acid Under Simulated Physiologic Conditions.* (30599)

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Evidence has accumulated that tumors of the bladder in persons engaged in manufacture of certain aromatic amines may result from *in vivo* formation of hydroxy derivatives

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from such compounds. Since these tumors are similar at pathologic examination to spontaneous tumors found in patients in the general population, the hypothesis has been advanced that spontaneous tumors of the bladder result from abnormal *in vivo* production of similar hydroxy derivatives from dietary tryptophan [3-hydroxyanthranilic acid (3-HOA)]. If this hypothesis is correct and if such metabolites are stable in urinary solutions, then these patients should excrete urine with higher concentrations of ortho-aminophenolic derivatives of tryptophan than those in urine excreted by normal subjects. Some investigators(1,2) have found higher urinary levels of these derivatives in patients with tumors, but Price and associates(3) observed that only about one-half of their patients with tumors of the bladder excreted high urinary levels of 3-hydroxykynurenine, an ortho-aminophenolic derivative of tryptophan. Benassi, Perissinotto, and Allegri(4) observed high urinary levels of 3-HOA in patients with renal carcinoma, but not in patients with cancer of the bladder. In fact, they found lower urinary levels of 3-HOA in some of their patients than in normal subjects.

During a series of fluorometric analyses for 3-HOA in our laboratory, we observed a rapid rate of decomposition of the standard solution of 3-HOA. Since previous investigators have apparently not defined conditions under which 3-HOA is stable and since these conditions could affect the amount found in urine at the time of analysis, we have investigated the stability of 3-HOA in buffer solutions as it relates to pH, oxygen tension, and catalytic ion concentrations within the physiologic range.

Methods. Since urine containing 3-HOA may remain in the bladder for periods of at least 8 hours (overnight), the effect of variables in the buffer solution on the stability of 3-HOA was determined after this time period.

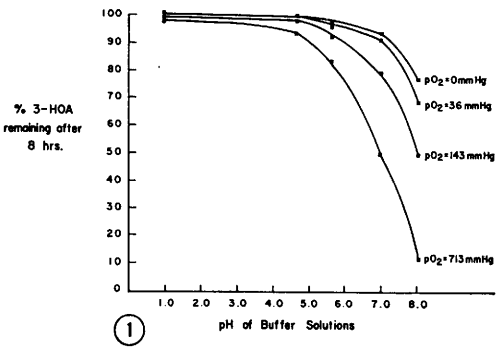
The effects of pH and pO_2 of the solution on the stability of 3-HOA were evaluated by incubation of 10 ml samples of buffer solutions containing 3-HOA (10.0 μg/ml) at pH of 1.0, 4.7, 5.6, 7.0, and 8.0 together at 37°C for 8 hours at one of the following partial pressures of oxygen: (a) $pO_2 = 0$ mm Hg, (b) $pO_2 = 36$ mm Hg, (c) $pO_2 = 143$ mm Hg, and (d) $pO_2 = 713$ mm Hg. This was accomplished by connecting flasks in series which contained the different buffer solutions and then forcing through them combinations of oxygen and nitrogen with the following proportions of oxygen: (a) 0%, (b) 5%, (c) 20%, and (d) 100%. At the beginning and end of the experimental periods, aliquots (0.1 ml) of the buffer solutions were added to 10 ml samples of phosphate buffer (pH 7.0, 0.1 M), and these solutions were analyzed for 3-HOA on the Aminco-Bowman Spectrophotofluorometer (320,415 mμ). Results are expressed as percentages of 3-HOA remaining after 8 hours.

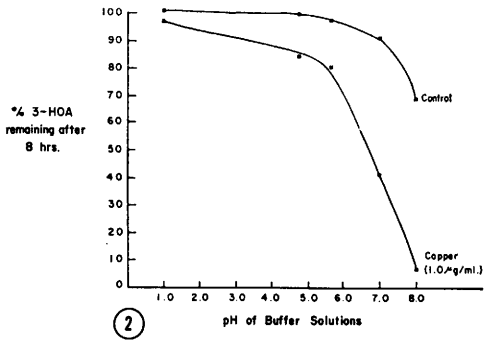
The effect of metal ions (Fe, Cu) on the stability of 3-HOA was determined by adding them (1.0 μg/ml) to the buffer systems and comparing the stability of 3-HOA in their presence and absence. These effects were determined at a pO_2 of solutions of about that found inside the bladder (36 mm Hg).

Results. Each point on the graph represents an average value obtained from triplicate experiments. Fig. 1 shows the effect of variations in pH and pO_2 of buffer solutions on the stability of 3-HOA. The stability of the acid is inversely related to these 2 variables in the physiologic pH range (4.7-8.0). At buffer pH approaching 1, 3-HOA became increasingly stable; at buffer pH greater than 1, the stability decreased steadily toward a buffer pH of 8.0.

Increasing the pO_2 of the buffer solutions ($pH > 1$) from about 0 mm Hg to 713 mm Hg gradually destabilized the acid; at buffer pH of 1, the stability was relatively unaffected by increasing the pO_2 of solution.

The catalytic effect of cupric and ferric ions (1.0 μg/ml) on the oxidative decomposition of 3-HOA is shown in Fig. 2 and 3 respectively. When the catalytic effect of cupric ion is added to the inherent instability of 3-HOA at buffer pH 8, only about 7% of the added 3-HOA can be recovered, as compared to about 68% in the control solution. The catalytic effect of cupric ion appears to be continuous throughout the physiologic range





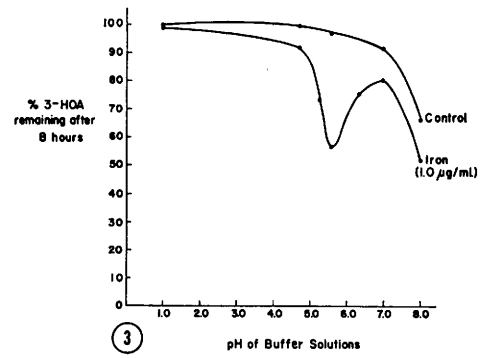


FIG. 1. Percentage of 3-hydroxyanthranilic acid (10.0 µg/ml) remaining after 8 hr in buffer solutions (0.1 M, 37°C) at different values of pH and pO_2.

FIG. 2. Catalytic effect of copper (1.0 µg/ml) on decomposition of 3-hydroxyanthranilic acid (10.0 µg/ml) in buffer solutions (0.1 M, 37°C) at pO_2 of 36 mm Hg.

FIG. 3. Catalytic effect of iron (1.0 µg/ml) on decomposition of 3-hydroxyanthranilic acid (10.0 µg/ml) in buffer solutions (0.1 M, 37°C) at pO_2 of 36 mm Hg.

of pH, whereas that of ferric ion is most prominent in acid solution (Fig. 3). At buffer pH 5.6, 3-HOA is only about 59% as stable in solution with ferric ion (1.0 µg/ml) as without it; at buffer pH 7.0 and 8.0, the inherent instability of the acid is almost unaffected by the addition of ferric ion.

Discussion. The data from these *in vitro* experiments suggest that significant amounts of urinary 3-HOA may decompose during its retention (8 hours) inside the bladder. Although 3-HOA is relatively stable in acid buffer solutions (pH 4.7-5.6) at pO_2 found inside the bladder (36 mm Hg, Fig. 1), the presence in acid urine of ferric or cupric ions (Fig. 2 and 3) would accelerate its rate of decomposition. In neutral or alkaline urine, 3-HOA would decompose spontaneously. At most physiologic pH values in urine, 3-HOA may therefore be unstable. Because of the effect of variables studied here and perhaps of other undefined ones (unidentified urinary oxidants), concentration of 3-HOA determined by present methods of urinary analysis may be an unreliable index of the true amount of 3-HOA formed metabolically by the subject.

Perhaps by the oral administration of antioxidants such as ascorbic acid and the acidification of urinary specimens to pH of 1 (immediately after the subject voids) urinary 3-HOA can be stabilized, and the true amount formed can be determined.

Some investigators(1,2) have found high urinary levels of 3-HOA in patients with tumors of the bladder; yet Benassi, Perissinotto, and Allegri(4) observed lower urinary levels of 3-HOA in some of their patients than in normal subjects. Perhaps all patients with tumors of the urinary bladder produce unphysiologic amounts of 3-HOA but because of variable urinary factors (pH, ionic catalysts, oxidants) they excrete normal or lower urinary levels occasionally, as a result of its decomposition within the bladder.

The possible decomposition of 3-HOA within the bladder raises the question of the significance of the decomposition products in the etiology of tumors of the bladder. Qualitative experiments in our laboratory on some of the products formed in buffer solutions from 3-HOA under simulated physiologic conditions have tentatively identified them to be compounds identical with or similar to those described by Butenandt(5). The possible car-

cinogenic activity of these products is now under investigation.

Summary. The stability of 3-hydroxyanthranilic acid (3-HOA) in buffer solutions was investigated as it relates to pH, oxygen tension, and catalytic ion concentrations. The data show that 3-HOA is unstable under certain simulated physiologic conditions, and suggest that the amounts found in some samples of voided urine are not necessarily the true quantities formed, but rather may be the amounts remaining after decomposition of the metabolite in the interim between formation and analysis.

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1. Boyland, E., Williams, C. D., Biochem. J., 1956, v64, 578.
2. Alifano, A., Papa, S., Tancredi, F., Elicio, M. A., Quagliariello, E., Brit. J. Cancer, 1964, v18, 386.
3. Price, J. M., Brown, R. R., Acta Un. Int. Cancer, 1962, v18, 684.
4. Benassi, C. A., Perissinotto, B., Allegri, G., Clin. Chem. Acta, 1963, v8, 822.
5. Butenandt, A., Keck, J., Neubert, G., Ann. Chem., 1957, v602, 61.

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Leukokinetic Studies. XII. Kinetic Studies of Normal Isologous Neutrophilic Granulocytes Transfused into Normal Subjects.* (30600)

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The clinical usefulness of erythrocyte transfusions is well established. In addition, understanding of the pathogenesis of the many types of anemia has been materially advanced by the development of methods for measuring red cell mass and red cell survival. Thus, infusion of labeled erythrocytes into a patient's own circulation (autologous study) or into the circulation of a compatible donor (isologous study) has made it possible to determine whether erythrocytes with a shorter than normal survival are themselves defective (intracorpuscular defect) or are subjected to an abnormal environment (extracorpuscular defect). Recently, granulocyte transfusions have also been employed both with the hope of clinical benefit and to study mechanisms of granulocytosis and granulocytopenia. For example, Yankee *et al* have reported clinical improvement in infected neutropenic patients following transfusion of granulocytes from normal donors(1). Similar results have been obtained in neutropenic patients transfused with granulocytes from donors with chronic myelocytic leukemia(2). Krill *et al*(3) have attempted to define intracorpuscular and extracorpuscular granulocyte defects in a patient with idiopathic neutropenia by comparing the kinetic results of isologous and autologous granulocyte infusions. We(4) and others(5) have attempted similar studies in patients with chronic myelocytic leukemia.

In an earlier study(6) 10 normal male subjects were transfused with isologous blood and it was observed that in 2 of the subjects the granulocytes disappeared at an accelerated rate. The present study was designed to extend these earlier studies by comparing transfused isologous and autologous granulocytes labeled with radioactive diisopropylfluorophosphate (DFP^{32}) with respect to their distribution in the vascular system at completion of the transfusion, their subsequent disappearance from the blood and their ability to migrate from the blood and enter an inflammatory exudate. A preliminary report of a portion of these data has been published(7).

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