the pitressin and extra water were stopped. This resulted after 6 hours in a copious diuresis, the patients usually reaching a new weight level below the initial figure.

A total of 20 observations or studies were made on the 6 subjects. During the water retention phase of each period of observation a reduction in the number and severity of asthmatic attacks occurred in 18 instances, 7 of which showed complete cessation of symptoms. In one case the symptoms remained unchanged and in another child who developed diarrhea a slight increase in symptoms occurred. During and after the diuretic phase improvement in symptoms continued in 19 instances with complete remissions developing 14 times. In the case which had diarrhea the asthma continued to be slightly aggravated during diuresis but improved during the subsequent period. In 6 instances symptoms recurred spontaneously after the end of the period of observation, while the remainder had remissions until upset by some extraneous factor. In one case 4 g of sodium chloride were administered after 10 days, which were free of asthma, all other factors remaining constant, and the asthmatic symptoms reappeared promptly. The results of the experiments are summarized in Table I.

From the foregoing it would appear that bronchial asthma can be ameliorated even in the presence of excessive hydration if there is an associated depletion of sodium chloride. This preliminary group of observations suggests that the sodium ion may exercise an adverse influence on the asthmatic patient, independent of its usual relation to hydration.

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Studies on Enzymatic Formation and Destruction of Uric Acid in Mammalian Blood.

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The colorimetric estimation of uric acid by a phosphotungstic acid reagent is made more specific by the use of uricase. The uricase preparation used consists of ox-kidney which has been acetone-dehydrated, benzene-defatted, air-dried, and finely ground. In the studies described here we used the direct colorimetric method[1] that

[1] Koch, F. C., *Practical Methods in Biochemistry*, 2nd edition, Wm. Wood, Baltimore (1937), 130, 284.

has been in use in this laboratory for a number of years. The cyanide solution described in this method is replaced by a solution of 2.5% sodium cyanide in 25% urea, and the tubes are kept at room temperature for 3 hours instead of being heated to allow the color to develop. Two-cc quantities of blood are incubated for 2 hours at 40° to 48°C. with 50-mg samples of uricase, after which the blood proteins as well as the uricase are precipitated with tungstic acid. The uric acid color-value of this filtrate is determined and subtracted from the value obtained on a filtrate from untreated blood—the difference represents true uric acid.

The average value obtained on 25 samples of human blood before uricase action was 3.0 mg % (varying from 1.9 to 4.6) and the average value of these same samples treated with uricase was 0.8 mg % (from 0.7 to 1.1). The true uric-acid value for these samples, then, is 2.2 mg % (varying from 1.1 to 3.8). Recoveries on 15 samples of human blood to which uric acid had been added in quantities of from 2 to 16 mg % averaged 92% (varying from 82 to 100%). The uric acid added to blood was quantitatively destroyed by uricase, leaving the same non-uric acid color-value as that of the original blood.

A typical sample of dog blood gave the following results: The uric-acid color-value on the untreated blood was 2.1 mg % and on the uricase-treated blood, 1.8 mg %, giving 0.3 mg % as the true uric-acid value. The true uric-acid value obtained on 10 samples of dog blood ranged from 0 to 0.5 mg %. Uric acid added to dog blood is recovered with the same accuracy as with human blood, and is quantitatively destroyed when uricase is allowed to act on it, showing that there is nothing in dog blood to prevent the detection of uric acid by this method.

The blood of white normal adult rats has a uric-acid value comparable to that of human blood when it is first drawn, but this value rises slowly as the blood stands at refrigerator temperature, and more rapidly when it is heated at 40° to 48°C. Uricase destroys the uric acid present in the original blood and that formed on incubation of the blood. This formation of uric acid was observed with blood drawn and kept sterile as well as with blood collected without any attempt to use sterile procedures.

A typical experiment showing the increase in uric acid as rat blood stands at refrigerator temperature follows: A pooled sample of blood was drawn by heart puncture from 10 adult normal rats. Oxalate was used to prevent coagulation. Immediately after the last sample was drawn which was about one-half hour after the

first sample, the blood contained 1.5 mg % uric acid before uricase action and 0.7 mg % after uricase action, making a true uric-acid value of 0.8 mg %. After the blood had stood for 2 hours this value had risen to 2.2 mg % (2.7 mg % before uricase and 0.5 mg % after uricase action). After 7 hours, the true uric-acid value was 2.5 mg %; after 26 hours, 3.4 mg %; after 4 days, 5.9 mg %; and the final analysis after 8 days showed a uric-acid content of 7.6 mg %. The non-uric-acid color-value in all the cases was from 0.4 to 0.6 mg %.

Different samples of blood vary in their original uric-acid content, but all show approximately the same increase in uric acid with time. This increase is from 2.5 to 3.0 mg % in 24 hours and 7.0 mg % in 8 days at refrigerator temperature. Blood incubated for 2 to 3 hours at 40° to 48°C shows a formation of uric acid of the same order as that produced in the blood standing 24 hours in the refrigerator. The production of uric acid is not observed when the blood proteins are precipitated, the uric-acid content of the filtrates remaining the same for the duration of the experiment.

In an attempt to explain the formation of uric acid on incubation of rat blood, the blood was incubated with adenine, guanine, hypoxanthine, and xanthine. Both guanine and xanthine caused a tremendous uric-acid formation. A typical experiment follows: Rat blood that had stood at room temperature for 4 hours after it was drawn showed a uric-acid content of 5.7 mg %, and after incubation at 40° to 48°C the value increased to 8.5 mg %. Since all the purines used were dissolved in alkali it was necessary to run a control with alkali. The value in this case was 9.2 mg %. The uric acid value of blood incubated with adenine was 7.2 mg %; with hypoxanthine, 7.3 mg %; with guanine, 32 mg %, and with xanthine, 40 mg %. In every case the uric acid produced was completely destroyed by uricase, the non-uric-acid color-value for this experiment being 1.1 mg %. The incubation of blood and uricase with xanthine or guanine either prevented the formation of uric acid or destroyed it as fast as it was formed. In each case in this experiment, 1 cc of blood was incubated with 1 mg of the purine. In this instance 1 cc of blood formed 0.2 mg of uric from 1 mg of guanine and 0.3 mg uric acid from 1 mg of xanthine in 4 hours at 40° to 48°. When 0.1 mg of hypoxanthine is incubated with 1 cc of blood, it also is converted into uric acid.

Neither human nor dog blood showed any tendency to form uric acid on incubating the blood alone, the values on these bloods remaining constant for a period of 5 days at refrigerator temperature.

No uric acid was formed by incubating these bloods with the purines used with rat blood.

Rat blood then contains enzymes which convert guanine, xanthine, and hypoxanthine into uric acid. The nature of the action suggests the presence of guanase and a very active xanthine oxidase.

Dixon and Keilin[2] reported the inhibition of xanthine oxidase by cyanide. The production of uric acid on incubation of rat blood alone or with guanine or xanthine was inhibited by 0.01 M potassium cyanide. It was of interest to find that the quinimine form of para-amino-phenol reported by Bernheim and Bernheim[3] as an inhibitor for xanthine oxidase also inhibited the formation of uric acid from xanthine by rat blood.

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Vitamin C and Peptone Shock in Dogs.

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Hochwald[1] and others have reported that the administration of Vitamin C to sensitized guinea pigs exercises a protective effect against the anaphylactic reaction produced by the provocative injection of the antigen. Hochwald reported that Vitamin C administration had no protective effect against histamine shock and therefore postulated that the mechanism of the action of Vitamin C in anaphylaxis was to prevent the liberation of histamine rather than to inhibit or inactivate the action of histamine after its liberation. Ungar, Parrot, and Levillain[2] reported some *in vitro* experiments tending to confirm this hypothesis. We were unable to confirm the protective effect of Vitamin C against anaphylaxis in the dog. Since the anaphylactic experiment is, however, quite complex and it is not always possible to discriminate between those agents which may interfere with the antibody-antigen reactions and those which mod-

2 Dixon, M., and Keilin, D., *Proc. Roy. Soc. London*, 1936, **119**B, 159.

3 Bernheim, F., and Bernheim, M. L. C., *J. Biol. Chem.*, 1938, **123**, 307.

1 Hochwald, A., *Z. f. d. ges. exp. Med.*, 1935, **97**, 433.

2 Ungar, G., Parrot, J. L., and Levillain, A., *C. R. Soc. de biol.*, 1937, **125**, 1015.