cortical extracts[2, 3,4] and by pure adrenal cortical steroids such as desoxycorticosterone[4, 6], corticosterone[7] and compound E[7]. Adrenalectomized mice may also be protected by adrenal cortical extract[6], desoxycorticosterone[5, 6] and progesterone[5]. We have used this test of sensitivity of adrenalectomized rats to low temperatures to demonstrate material in the urine of normal men which appears to be related in its biological action to the adrenal cortical steroids. We are not prepared at this time to say whether this biologically active material is specifically an adrenal cortical steroid metabolite. Our experience thus far, however, suggests that this may be true.

Cortin-like action of extracts of urine from various types of patients has been reported by Anderson, Haymaker and Joseph[8] and by Weil and Browne[9, 10]. These investigators studied urine from patients with Cushing's syndrome, hypertension, chronic osteomyelitis, as well as post-operative patients who had no symptoms of shock. The former group of investigators used the maintenance of the adrenalectomized rats as their means of demonstrating biological activity, while the latter group used the technique described by Selye and Schenker[3] involving the sensitivity of the adrenalectomized rats to cold. Perla and Marmorstein-Gottesman[11] reported the presence of a benzene soluble material in human urine which was capable of increasing the resistance of adrenalectomized rats to histamine, while Grollman and Firor[12] reported that benzene extracts of human urine were capable of maintaining adrenalectomized rats.

The urines of three normal men, 26, 26 and 31 years of age, respectively, were collected over a three-day period. The fresh urine was extracted three times with ethylene dichloride at room temperature. For each extraction one part of ethylene dichloride to four parts of urine was used. The ethylene dichloride extracts were evaporated to dryness *in vacuo,* taken up in small volumes of absolute ethanol and again evaporated to dryness. This procedure was repeated twice. This technique is similar to that previously employed by Weil and Browne[10]. The final dry, brownish oil was taken up in 10 per cent. ethanol and administered by stomach tube to adrenalectomized rats weighing 35 to 45 grams. The rats were adrenalectomized 24 hours before the assay was run. The details of the assay technique will be described in another report.

[6] Unpublished experiments.

[7] E. C. Kendall, *Jour. Am. Med. Assn.,* 116: 2394, 1941.

[8] E. Anderson, W. Haymaker and M. Joseph, *Endocrinology,* 23: 398, 1938.

[9] P. Weil and J. S. L. Browne, SCIENCE, 90: 445, 1939.

[10] P. Weil and J. S. L. Browne, *Proc. Am. Physiol. Soc.,* 121: 652, 1939.

[11] D. Perla and J. Marmorsten-Gottesman, *Proc. Soc. Exp. Biol. and Med.,* 28: 1024, 1931.

[12] A. Grollman and W. W. Firor, *Proc. Soc. Exp. Biol. and Med.,* 30: 669, 1932–3.

The results on the urines collected from three normal men are represented in Table 1. It is seen that the

TABLE 1

RESPONSE OF ADRENALECTOMIZED ANIMALS (EXPOSED TO 5° C.) TO EXTRACTS, FROM THE URINE OF NORMAL MEN

Subject		Urine collection Days	Administered per rat extract equivalent		Number of rats	Mean survival Hours	Increase in mean survival Per cent.
Number	Age		cc	Hours			
–	—	–	0	0	9	5.9	—
1.	26	3	295	6.0	8	8.7	48
2.	26	3	194	5.8	10	8.1	37
3.	31	3	236	6.0	9	8.9	51

equivalent of 6.0, 5.8 and 6.0 hours of urine respectively produced increases in survival time of 48 per cent., 37 per cent. and 51 per cent., respectively. When these increments are compared to the responses found for a Wilson Adrenal Cortical extract, it is found that the urine contains cortin-like material equivalent to 0.15 to 0.18 cc of extract per day.

In addition to the experiment described above, it has been possible to demonstrate cortin-like activity in the extracts of composite samples of normal male urine and in the urine of post-operative male patients, but we have been unable thus far to detect such activity in the urine of patients with Addison's disease.

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HAY FEVER AND VITAMIN C

DURING the past four years one of us made occasional observations indicating a lowering of the body level of vitamin C during hay-fever attacks.

HISTAMINE THEORY

Other workers developed the interesting theory that histamine, $C_5H_9N_2$, although a normal constituent of the blood, is thrown into the blood stream in excessive amounts during allergic attacks and that this excess histamine is responsible for some of the unpleasant symptoms. On the assumption that histamine might react with vitamin C, or ascorbic acid, we mixed water solutions of the two substances but observed no reaction in absence of free oxygen. Upon bubbling a slow stream of air through the solution it was easy to detect evolution of ammonia. Titration with 2,6-dichlorophenol-indophenol showed loss of vitamin C. Later we learned that this reaction was already known. Since there is a little dissolved oxygen in blood serum, the body furnishes the proper conditions for very slow reaction.

REQUIRED REPLACEMENT OF VITAMIN C

A low level of vitamin C in the body causes weakness as well as other ills, so it is apparent that losses due to hay fever should be made good by a diet extremely rich in this vitamin, or even, in severe attacks, by administration of the pure synthetic product.

Many tests of this idea have been made by others, but there has been much confusion as to the dosage of ascorbic acid required for relief.

We therefore experimented[1] with twenty-five hay-

[1] We are in debt to Dr. H. A. B. Dunning, of Baltimore, for generous support of this research.

TABLE 1

THE RESULTS GIVEN IN TABLE 1 ARE SIGNIFICANT. THE SIGN "——" MEANS THERE WAS NO DETERMINATION OR DOSAGE FOR THAT COLUMN

Patient number	Vitamin C in 24-hour urine before dosage	Excretion of C after one week of 100 mg daily	Symptoms after one week of 100 mg daily	Symptoms after week of 200 mg daily	Symptoms after dosage of 500 mg daily
1	49 mg	——	——	"Immense improvement." Gain after two days	——
2	8 mg	218 mg ? (after dose of 200 mg daily)	No relief	Distinct gain	"No hay fever" after 3 days
3	8 mg	1–6 mg (after 2 days of 600 mg rose to 96 mg)	No relief	——	"Much better" after 2 days
4	42 mg	90 mg	No relief	Slight gain	"Almost no hay fever" after 4 days
5	20 mg	35 mg	Some relief	Decided relief	"Almost no hay fever" after 2 days
6	11 mg	73 mg	No relief	No relief	"Hay fever practically gone" after 3 days
7	6 mg	1–6 mg (after 3 days of 500 mg rose to 102 mg)	Some relief	——	Great relief after 3 days
8	16 mg	(after 12 days rose to 221 mg)	——	Great relief	——
9	——	——	Little relief	Great relief	——
10	——	——	Little relief	Great relief	——
11	——	——	Little relief	"Felt fine"	——
12	——	——	——	"Better physical condition"	"Highly favorable reaction. More refreshing sleep" after 2 days
13	16 mg	160 mg ?	No relief	——	"Distinctly better" after 3 days
14	——	——	——	Great relief in few days	——
15	——	——	——	"Much less tired"	——
16	——	——	Definitely improved	——	——
17	9.5 mg	105 mg	Some relief	——	——
18	——	——	"It helped"	——	——
19	——	——	——	Began in July. "No hay fever at all—after years of suffering"	——
20	0–5 mg	67 mg (after 10 days of 200 mg daily)	——	Vast improvement. From weakness to vigor	——
21	——	——	——	——	(Invalid from asthma.) After 1 week became astonishingly vigorous and healthy
22	High	Higher	No relief	No relief	Little relief
23	63 mg	118 mg ?	No relief	——	No relief after 3 days
24	10 mg	——	Broke out in rash and quit	——	——
25	——	——	——	——	1000 mg gave great relief the next day

fever sufferers in Oberlin at three levels of vitamin intake; 100 mg, 200 mg and 500 mg daily, administered during the ragweed season from August 15 to September 15.

URINARY EXCRETION

In most instances we were able to determine the 24-hour urinary excretion[2] of vitamin C before giving the first dose. Whenever possible we determined the daily excretion again after one week. It is the general opinion that a healthy individual of average weight excretes from 30 to 50 mg of vitamin C daily in the urine.

With ordinary methods of collection we have observed considerable loss by oxidation, so we used the very simple but effective method previously devised by Holmes and Campbell.[3]

The maximum pollen count in Cleveland, thirty-five miles from Oberlin, averaged about 87 for the last half of August and about 80 for the first half of September. "Sneezing begins at 15." Oberlin, away from Lake Erie, has more pollen than Cleveland.

The initial daily vitamin C excretion of twelve patients (including three not charted) averaged only 10 mg, indicating a very low level, due to destruction or inactivation of the vitamin. One excreted 20 mg, three were satisfactory and the others were not measured.

Usually, after a week of treatment the excretion rose to excellent levels, in some instances indicating body saturation. Strangely enough, patients No. 1 and No. 4 showed very good vitamin C levels before starting treatment, yet they were greatly benefited by adequate dosage.

It is evident from the table that only five sufferers made a noticeable gain in health after a week of 100 mg daily dosage, while twelve gained decidedly after a similar period of 200 mg dosage and eight reported remarkable improvement after three or four days at the 500 mg level. One got almost immediate relief after a single dose of 1,000 mg. Apparently there was distinct gain with 88 per cent. of the patients.

TREATMENT

We strongly recommend that pharmaceutical firms prepare 250 mg tablets of vitamin C (or capsules to be emptied on the tongue) in order to lower the cost and to simplify dosage. The patient (after consulting the family physician, as was done in our own recorded experiments) would do well to begin with a daily 250 mg dose and, if no decided improvement results after one week, to try 500 mg daily until satisfactory progress is observed. After that he might get along comfortably on 250 mg or less during the season.

Since excess vitamin C is excreted rapidly in the urine, it is impossible to go beyond body saturation. Rarely are any irritating effects observed, yet one of our patients reported development of a rash.

REDUCING ACIDITY FOR SENSITIVE PATIENTS

Patients objecting to the acidity of ascorbic acid are advised to mix with the vitamin an amount of baking soda nearly equivalent chemically. If the vitamin is visibly crystalline, equal volumes of vitamin and sodium bicarbonate are used; if the vitamin is in a fluffy powder form, about one third that volume of sodium bicarbonate will serve. It is a mistake to mix water solutions to be kept for days, as oxidation occurs rapidly in the neutralized vitamin solution. We proved, by tests on several people, that after keeping a mixture of the dry powders eight hours and then administering there was no apparent loss of the vitamin. Patients with gastric ulcer, usually on a diet low in vitamin C because of difficulty with the roughage of vegetables and the acidity of fruits, may profit by the observation above.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLIFIED PROCEDURE FOR THE CONCENTRATION AND PURIFICATION OF INFLUENZA VIRUS[1]

THE observations of Hirst[2] and McClelland and Hare[3] have clearly demonstrated that influenza virus in chorioallantoic fluid of the chick embryo can be directly adsorbed by the erythrocytes of the embryo. Hirst[4] has also shown that the adsorbed virus can be readily eluted from the red blood cells at temperatures of 22° to 37° C. In addition, these investigators have pointed out[5,6] that the precipitate which forms when

[2] Miss Jean Risinger assisted us with some of the analytical work.

[3] Harry N. Holmes and Kathryn Campbell, *Jour. Lab. Clin. Med.*, 24: 1293, 1939.

[1] These investigations were aided through the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, United States Army.

[2] G. K. Hirst, SCIENCE, 73: 335, 1941.

[3] L. McClelland and R. Hare, *Canadian Public Health Jour.*, 32: 530, 1941.

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[5] G. K. Hirst, E. R. Rickard and L. Whitman, *Proc. Soc. Exp. Biol. and Med.*, 50: 129, 1942.