(5γ/ml) is added with 10 ml KH_2PO_4-NaOH buffer solution (pH 7) and 5 ml CNBr solution, and heated in a water bath at 70°–80° C for 8 min, cooled in ice-cold water, and 5 ml 15% NaOH solution added.

Calculation. Let a = content of nicotinamide in the sample for determination; b = content of nicotinamide in the sample of intermediate standard; c = amount of nicotinamide added to the sample for determination; f = dilution factor; and r = recovery (%) of the added nicotinamide throughout the operation. Then

$$\text{Nicotinamide } (\gamma/\text{g}) = \frac{acf}{b-a}$$

$$\text{Recovery } (\%) = \frac{b-a}{c} \times 100.$$

KH-4B is a carboxylic-type cation exchange resin prepared from phenoxyacetic acid and formaldehyde, and has a total capacity of 5.84 mEq/g dry resin. The same types of cation exchange resins, Amberlite IRC-50 (Rohm & Haas Co.) (*4*) and Wofatit C (I. G. Farbenindustrie Akt.-Ges.) (*5*) may be suitable for use in place of KH-4B. Amberlite IRA-400 is a strong-base–type anion exchange resin, and has a total capacity of 2.5 mEq/g dry resin. Since the chemical characteristics of Amberlite IRA-400 are analogous to those of sodium hydroxide, carbonate-free reagents and distilled water must be used for the treatment.

Cationic impurities in an extract solution are adsorbed by the filtration through KH-4B-Na (sodium salt–type of KH-4B) at pH 5, but nicotinamide is not adsorbed by such an operation followed by the rinse with hot distilled water. Anionic impurities in an extract solution are adsorbed by filtration through Amberlite IRA-400-OH (hydroxide-type of Amberlite IRA-400) at pH 5, but nicotinamide is not adsorbed by such an operation followed by the rinse with distilled water.

Nicotinamide contents of the pupae and eggs of various types of silkworm *B. mori* were determined by the method described above, and the analytical results shown in Table 1 were obtained.

TABLE 1

NICOTINAMIDE CONTENT OF SILKWORM

Material	Nicotinamide content (γ/g)	Material	Nicotinamide content (γ/g)
Pupa of White-1 type	59	Egg of White-1 type	111
" " White-2 "	13	" " White-2 "	30
" " normal "	60	" " normal "	113

By Chaudhuri-Kedicek's method, kynurenine in the White-1 type mutant (*6*) and 3-hydroxy kynurenine in the White-2 type mutant (*6*) gave green and yellowish-green fluorescence, respectively, and unknown substances other than nicotinamide in each type of *B. mori* gave yellowish fluorescence after treatment with cyanogen bromide. Therefore the fluorescence of nicotinamide was greatly contaminated and the estimation was almost impossible. But in our method these contaminating fluorescences were eliminated completely by the use of KH-4B-Na and Amberlite IRA-400-OH, and the estimation of nicotinamide was performed without difficulty.

The new determination method of nicotinamide by use of synthetic ion exchange resins as described should be especially useful in the investigation of tryptophan metabolism in future.

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The Use of K^{42}-tagged Erythrocytes in Blood Volume Determinations[1]

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Erythrocytes have been tagged with $Fe^{55, 59}$ (*1*), P^{32} (*2, 3*), and Cr^{51} (*4*) and used for blood volume determinations by the *in vivo* dilution technique. Radiopotassium (K^{42}) has two properties that make it useful for tagging erythrocytes for special types of experiments. First, it decays by emission of energetic β-particles (3.6 and 2.0 mev) and γ-rays, which are easily detectable in liquid samples. Therefore extremely small amounts (less than 2 μc) may be used effectively. A second advantage is that it has a short physical half-life of 12.44 hr. Consequently, blood volume determinations or other studies may be repeated at relatively short intervals without concern for residual activity or hazards created by the concentration of radioisotopes in any part of the body. Furthermore, isotopes such as P^{32} and I^{131}, with appreciably longer half-lives, may be administered shortly thereafter. Because of the great difference in half-lives, the activity of the longer-lived isotope in body fluids can be determined after the K^{42} has decayed, without the need for complicated corrections.

This paper describes experiments in which comparison studies were made between almost simultaneous blood volume determinations with erythrocytes labeled with K^{42} and P^{32}. The *in vivo* half-life of the circu-

[1] Reviewed in the Veterans Administration and published with the approval of the chief medical director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or the policy of the Veterans Administration.

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lating K^{42}-labeled cells was also determined. The K^{42} and P^{32} (14.3-day half-life) were obtained from Oak Ridge. Each isotope was prepared for human injection in neutral sterile isotonic saline solution.

Ten ml of heparinized blood was incubated with about 50 μc carrier-free P^{32}, and a similar volume was incubated with 100–200 μc K^{42} contained in about 50 mg of stable potassium carrier. With this carrier level, the presence of serum potassium had no effect on the uptake of potassium by the cells. The method of incubation and preparation of the blood for injection was a modification of the method described by Reeve and Veall (*5*).

The percentage of K^{42} taken up by the red blood cells ranged from 2% to 6% under varying conditions in which approximately the same amount of carrier potassium was used. The factors determining the rate of uptake are at present under study.

A weighed volume of the saline-suspended K^{42}-labeled cells was injected, and heparinized blood samples were withdrawn about 5 and 15 min later. This procedure was then repeated with the P^{32}-labeled cells. The radioactivity of the K^{42} in the injected and withdrawn samples of blood was determined immediately. The radioactivity of the P^{32} in the injected and withdrawn samples was determined 5 days later, at which time the K^{42} in the withdrawn blood was no longer detectable. The blood volume in each case was calculated in the usual manner. For the determinations of the biological half-life of the K^{42}-labeled cells, multiple specimens were taken for a 12- to 15-hr period.

The results of the almost simultaneous blood volume studies are given in Table 1. These two determinations

TABLE 1

Blood volumes

Patient	K^{42} (ml)	P^{32} (ml)	Av (ml)	Difference from av (%)
F. S.	5,940	6,400	6,170	3.7
J. R.	7,655	7,800	7,727	0.9
J. P.	4,945	4,820	4,882	1.3
O. H.	4,170	4,080	4,125	1.1
R. F.	4,750	4,870	4,810	1.3
J. B.	5,950	5,300	5,625	5.7

give essentially the same values within the errors inherent in the methods.

The biological half-life of the K^{42}-labeled cells *in vivo* ranged from 28 to 35 hr. This is consistent with studies on the *in vitro* rate of potassium uptake in red blood cells (*6*) and with studies that we will report elsewhere on the *in vivo* uptake of K^{42} by the RBC after plasma specific activity has reached a constant.

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The Metabolism of *Blastomyces dermatitidis*, Antagonists to the Growth-inhibiting Effect of Trimeton Maleate[1]

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Previous reports from this laboratory (*1, 2*) presented evidence that trimeton maleate[2] (1-phenyl-1-α-pyridyl-3-dimethylaminopropane maleate, Schering) could completely inhibit the growth of *Blastomyces dermatitidis*[3] and to a lesser extent the growth of other fungi, which results are similar to those reported by Carson and Campbell (*3*) using different antihistaminics. In our series (*2*) partial inhibition of *B. dermatitidis* was evident with 0.0003 *M* trimeton maleate, with complete suppression of growth of the fungus at 0.015 *M* concentration.

For investigation of the therapeutic implications of these findings, mice were infected with *B. dermatitidis* and treated with trimeton maleate. Twenty-eight albino mice of the Swiss strain (18–20 g) were infected by the intraperitoneal route with a heavy suspension of the yeast phase of *B. dermatitidis* suspended in 4% maltose, 1% peptone water. The organisms used for the mouse inoculations had been grown on blood agar in the incubator at 37° C for 2 weeks. The animals were divided into 2 groups for therapy, with paired controls receiving no treatment. Therapy was administered by subcutaneous injection of trimeton maleate, with a daily dosage of 40 mg/kg, divided into 2 injections/day. One group of the treated mice received therapy from the day of infection; in the other group therapy was initiated on the tenth day after the date of infection. For the prophylactic trial, 20 mice were divided into 2 groups. One group received 40 mg/kg trimeton maleate daily for 5 days preceding infection with *B. dermatitidis*; the other group received no pretreatment, and treatment was instituted at the time of experimental infection.

All animals were sacrificed on the twenty-first day after infection, since in the experience of Spring (*4*), infection with *B. dermatitidis* is at a maximum in mice at this time. At autopsy, infection could be readily demonstrated by the presence of widespread nodules and partly necrotic masses affecting the mesentery, the retrosplenic and retrohepatic regions. Infection was determined by positive wet mounts, positive cultures, and by demonstrating the microorganism in histological sections.

These experiments indicated that trimeton maleate,

[1] Preliminary report.

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