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Improved Urinary Excretion Test for Assay of Intrinsic Factor. II. Sensitive Counting Technic.* (22349)

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The measurement of urinary excretion of vit. $B_{12}Co^{60}$ ($B_{12}Co^{60}$) following oral administration is used in the diagnosis of pernicious anemia(1,2) and as an assay for intrinsic factor(3,4). The use of human beings as experimental subjects necessitates the use of $B_{12}Co^{60}$ of low activity.† Since Co^{60} is a gamma emitter, the instrument of choice for its measurement is the scintillation counter. Most present well-type scintillation counters however, cannot count more than a 10 ml sample and therefore the low activity present in a 24 hour urine sample presents a counting problem which can only be solved by excessively long counting or by concentration of the urine samples. Flat scintillation crystals, upon which a beaker of liquid can be placed, do not offer significantly greater sensitivity than the well-type scintillation counter. Weiner and Peterson(5) designed a G-M well counter for measuring the radioactivity in liquid samples contained in 3 liter bottles, whereas Baskin *et al.*(6) constructed a well-shaped scintillation counter with a 35 ml capacity.

Using an available small well-type scintillation counter, a beaker with a capacity of one liter was designed fitting into the well and surrounding the top and sides of the crystal. This paper reports on the results obtained with the use of this beaker.

Methods. The design of the scintillation counter‡ is shown on the left side of Fig. 1. The counter consists of an RCA 5819 phototube, a thallium activated sodium iodide crystal, 1¾ in. diameter and 1 in. thick with a hole ¾ in. in diameter and 1 in. deep, and a cathode follower preamplifier. A 4 ml sample can be counted in a screw-cap glass vial and the vial is then placed in the well separated by a brass sleeve to prevent contamination of the crystal housing. The beaker in counting position is shown on the right side of Fig. 1. It was constructed§ starting with a two liter beaker from which the bottom was removed. The new bottom enclosed the crystal on top and sides with a projection fitting downward into the well. The counter and beaker were enclosed in a lead shield 3 in. thick. The background was about 110 cpm.

Results. 0.5 μc of vit. $B_{12}Co^{60}$ was diluted to 1000 ml and increasing amounts of this solution were placed in the beaker and counted. The values reported in Table I are corrected for background. As is evident from Table I, the use of the beaker gives an increase in counting rate of approximately 40 times that obtained using the standard 4 ml vial. As one would expect, the efficiency of

* See Ref. 4 for Paper I in this series.

† The maximum permissible dose has been set at 3 μc by the Subcommittee on Permissible Internal Doses of the National Committee on Radiation Protection.

‡ Model S_1—Purchased from H. O. Anger, Berkeley, Calif.

§ Constructed by L. & K. Glass Co., Lyndhurst, N. J.

counting with the beaker (3.5%) is less than direct counting in the vial (22%), but the over-all gain due to the larger volume is much greater than the loss in efficiency. It is reasonable to expect that much greater sensitivity would result if the beaker was designed to fit over larger crystals such as the commercially available 1¾ in. diameter by 2 in. thick crystals.

Due to the geometry of this beaker and crystal a volume of 500-750 ml gave the greatest efficiency, since at this volume there is equal amounts of liquid equidistant from all surfaces of the crystal. The optimum method, though, is to count one liter of liquid if available.

The increase in sensitivity permits the use of $B_{12}Co^{60}$ of lower specific activity in the urinary excretion test. One subject can receive 12 doses of 0.25 μc instead of 6 doses of 0.5 μc therefore, allowing the use of the same patient for additional intrinsic factor assays. Furthermore, the use of the beaker requires less counting time with a low excretion of radioactivity and makes it unnecessary to concentrate the urine sample. Some of the advantages of the beaker over the vial methods are listed in Table II. Even at the 5.0% excretion value if the dose of $B_{12}Co^{60}$ administered were reduced to the same amount as permitted by the use of the beaker and if the urine were not evaporated it would be necessary to count 100 minutes in order to reduce the standard error of counting to below 5%.

TABLE I. Comparison of Sensitivity of Vial *vs.* Beaker Counting Methods.

Vol	C.P.M.	Activity in sample, μc	Efficiency, %	Beaker sensitivity ratio*
Vial				
4	978	.002	22	—
Beaker				
100	3,783	.05	3.4	3.9
200	7,832	.10	3.5	8.0
300	12,695	.15	3.8	13.0
500	21,769	.25	3.9	22.2
750	32,492	.375	3.9	33.3
1000	38,666	.50	3.5	39.8

* $\frac{\text{Efficiency} \times \text{volume of beaker}}{\text{Efficiency (22\%)} \times \text{volume of vial (4 ml)}}$.

The technic described in this report also appears applicable to other biological materials of large volume and low activity from gamma radiation. It would seem particularly suitable for counting $B_{12}Co^{60}$ in feces in

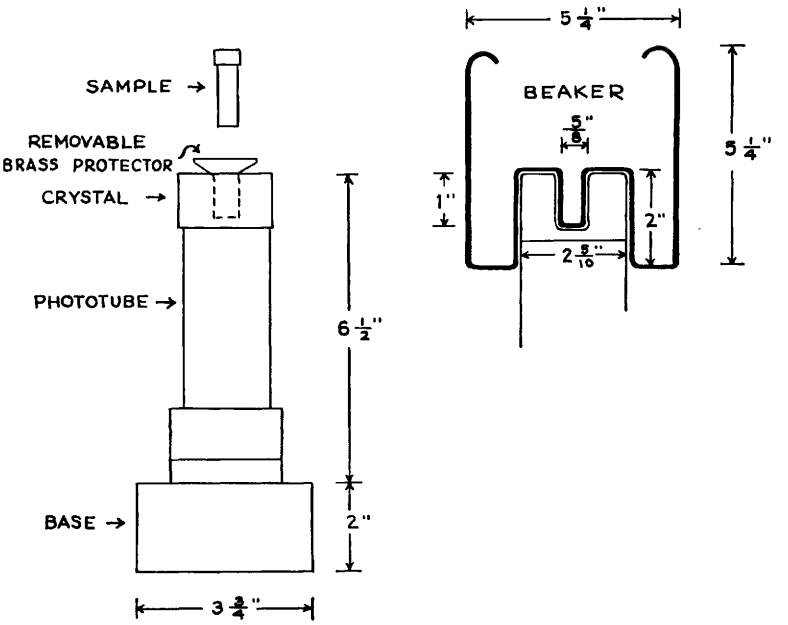

FIG. 1. Left: Well-type scintillation counter for measuring gamma radiation contained in a 4 ml glass vial. Right: Same counter showing design and position of a beaker for counting up to one liter of liquid.

TABLE II. Comparative Features of Vial and Beaker Counting Methods.

Excretion in 24 hr urine sample*	0.5%		5.0%	
	Vial	Beaker	Vial	Beaker
Dose of $B_{12}Co^{60}$ administered (μc)	.5	.25	.5	.25
Max permissible No. of doses/patient	6	12	6	12
Degree of concentration	10	None	10	None
Vol counted (ml)	4	1000	4	1000
C.P.M. corrected for background	49	97	490	970
Counting time† (min.)	20	9	1	1

* Assuming the 24 hr urinary vol to be 1 liter.
† Minimum necessary time so as not to exceed 5% stand. error of counting and assuming a constant background rate of 110 cpm.

the fecal excretion technic for the estimation of intrinsic factor activity(7).

Summary. Measurement of gamma radiation of low specific activity contained in relatively large volumes can be adequately performed with the use of a specially designed beaker which fits into the well and covers the cross section of a well-type scintillation counter. The increase in sensitivity obtained with this beaker allows the administration of smaller oral doses of vit. $B_{12}Co^{60}$ for additional assays using the same patient for the urinary excretion tests for intrinsic factor. In addition, no concentration of the urine sample is necessary and less counting time is required.

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Effect of Nalorphine on Gastric Emptying in Man. (22350)

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The consensus of most authorities is that morphine noticeably retards the emptying of the stomach in the human(1-3), as well as in the dog(4). Nalorphine, which antagonizes the narcotic effect of morphine when both are present, has certain actions when given by itself similar to morphine, but less intense. It is, therefore, of interest to determine the effect of nalorphine alone on gastric emptying.

Methods. The experiments were performed on 7 healthy medical students, 5 males and 2 females. The test meal consisted of 15 g of farina, cooked to a volume of 200 cc. One hundred g of barium sulfate were added so that the meal could be seen with the fluoroscope. The subjects ate this meal about 8:30 A.M. No food had been eaten since the previous evening. Determinations were made at weekly intervals. Fifteen minutes before they ate the meal, the subjects were given a subcutaneous injection of either saline (controls) or 5 mg of nalorphine hydrochloride* in saline. The time it took the meal to leave the stomach was determined fluoroscopically to the nearest 15 minutes.

Results. Table I shows that nalorphine delayed gastric emptying in all subjects, and more than 4 hours in 2. Only two showed a delay of less than 2 hours. All subjects, ex-

* We wish to thank Merck and Co. for the gift of nalorphine hydrochloride used.