

horse serum (diluted 1:4)/100 g of body weight.

Series 2. Ten guinea pigs were each pretreated by the i.p. injection of 2 mg D-catechol for 4 days. On the fifth day they each received, in addition, 1 ml of a 1:10 dilution of horse serum given subcutaneously. D-Catechol was continued for 13 subsequent days. On the fourteenth day, each animal was challenged by the i.v. injection of 1 ml of horse serum. A simultaneous control group was treated identically except for the D-catechol administration.

In vitro inhibition. D-Catechol effectively inhibited the action of the histidine decarboxylase present in the guinea pig kidney extract. The reaction mixture without the D-catechol showed appreciable amounts of liberated histamine. The other controls were negative. The chromatogram is shown in Fig. 1.

In vivo inhibition. Series 1. As Table 1 indicates,

TABLE 1
RESULTS OF ANAPHYLACTIC CHALLENGE OF ACTIVELY
SENSITIZED (TO HORSE SERUM) GUINEA PIGS
TREATED WITH D-CATECHOL

Guinea pig No.	Series 1		Series 2	
	Control	Treated	Control	Treated
1	++++	++++	++++	++++
2	++++	+++	++++	++++
3	++++	+++	++++	++++
4	+++	++	++++	++++
5	+++	++	++++	++++
6	+++	++	++++	++++
7	+++	++	++++	++++
8	++		++++	++++
9	++		++++	++++
10	++		++++	++++

++++ Died.

+++ Markedly severe symptoms; collapse with eventual recovery.

++ Severe symptoms; marked respiratory distress.

3 of the control group of guinea pigs died in shock, 4 survived markedly severe anaphylactic symptoms, and 3 suffered severe reactions. Of the test group, 1 died in anaphylactic shock and 6 survived. Of the survivors 2 suffered markedly severe and 4 severe symptoms.

Series 2. All control and test animals died in anaphylactic shock.

There seems to be little doubt that D-catechol can inhibit the action of tissue histidine decarboxylase. However, these results confirm those of others (7, 9-11) that the flavonoid is without an appreciable *in vivo* action on the enzyme. Since in our hands the sensitization technique of Raiman, Later, and Necheles failed to produce uniformly fatal results in the control group of animals, we considered the results obtained in the treated group as equivocal. For this reason a sensitization procedure was adopted in which an LD₁₀₀ was employed. The treated animals in this group showed no resistance to the challenge. If D-catechol does have an *in vivo* inhibiting action on tissue histidine decarboxylase, the results suggest either that anaphylactic symptoms are produced by

some mechanism other than histamine release or that inhibition is not complete and at least a sufficient amount of histamine is formed to account for the symptoms. However, the severity of the reactions seems to indicate that D-catechol plays but a negligible role in preventing anaphylactic shock in the guinea pig, which presumably results from histamine release.

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Counting of Radioactivity in Liquid Samples

R. F. Goddu and L. B. Rogers¹

Department of Chemistry and
Laboratory for Nuclear Science and Engineering,
Massachusetts Institute of Technology, Cambridge

During the recent work using the counting technique proposed by Freedman and Hume (1) for liquid samples, two pitfalls were noted when the samples were not counted immediately. In the present study, aluminum cups were carefully coated with Chem-Lac Lacquer 117-2 (Chem-Lac Products, Inc., Cambridge, Mass.) and allowed to dry. The samples counted contained 10⁻³ M silver as carrier and 7.5-day Ag¹¹¹ as tracer in 0.8 M potassium thiocyanate solution. The surface of the sample was coated with thinned Chem-Lac Lacquer, as described by Freedman and Hume, and allowed to dry. At least 10,000 counts were taken on each sample to cut the statistical error of counting to 1%.

When 1-ml samples were counted in the coated aluminum cups, appreciable plating-out of silver took place within 24 hr, as evidenced by the substantial decrease in activity in Set 1, Table 1. The plating was also visible to the naked eye. After 2 more days, another complication was evidenced by a substantial increase in count, which more than compensated for the decrease due to plating.

Studies of this increase in counting rate were carried out in lacquered glass cups to avoid changes

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TABLE 1
CHANGE IN COUNTING RATE WITH AGE OF SAMPLE, CORRECTED FOR DECAY

Set	Description of sample	Relative counting rate on successive days						
		0	1	2	3	4	5	7
1	1 ml of solution in Al cup kept in air (av of 2)	100	73	—	—	82	—	—
2	1 ml of solution in glass cup kept in air (av of 3)	100	106	109	111	115	—	125
3	1 ml of solution in glass cups kept in hygrostat over 1 <i>M</i> KSCN (av of 4)	100	104	105	104	104	—	106
4	5 ml of solution in glass beakers kept in air (av of 2)	100	98	98	100	—	—	—
5	1 ml of solution in glass cups kept in hygrostat over water (av of 7)	100	100	100	—	—	100	99

TABLE 2
RELATIVE COUNT CORRECTED FOR DECAY AND FOR DECREASE IN VOLUME

Set	Description of sample	Relative counting rate on successive days					
		0	1	2	3	4	7
2	1 ml of sample in glass cups kept in air (av of 3)	100	101	99	99	99	92
3	1-ml samples in glass cups kept in hygrostat over 1 <i>M</i> KSCN (av of 4)	100	102	103	101	101	102

caused by plating. Adsorption onto the lacquered surface was not thought to be significant, because of the presence of carrier and complexing agent, an assumption substantiated by the experimental results described below. When samples were weighed immediately before or after they were counted, an observed count could be corrected by a factor that took into account the increase in concentration resulting from loss of solvent through the dry film of lacquer. Set 2 in Tables 1 and 2 illustrates typical results before and after multiplying by a correction factor which is the percentage of original volume remaining at the time the sample was counted. As one might expect, the evaporation can be eliminated by using a hygrostat, as shown by Set 5. Occasionally, small droplets of water condensed on top of the film, but they can be removed easily without damage to the film by using an absorbent paper tissue. Ordinarily, the change in weight of a cup having a surface area of approximately 3.5 cm² was about -30 mg/day in the open air, -6 mg/day over 1 *M* potassium thiocyanate, and ± 1 mg/day over water. One can also decrease the evaporation error by using a large volume of sample without changing the surface area, so that the relative loss in weight is insignificant, as illustrated by Set 4.

These studies point out that in spite of careful coating of a metal container the plating of a more noble metal onto a less noble metal is very probable. More important is the fact that either the counting of liquid samples must be done within a few hours after preparation of the sample or the loss of solvent eliminated by placing the samples in a hygrostat after the lacquer film has dried. For best results, a correction should be applied for changes in the volume of the sample. Use of a hygrostat appeared to increase the average lifetime of the lacquer films from about 10 days in open air to about 3 weeks.

Reference

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A Volumetric Microrespirometer for Studies of Tissue Metabolism¹

Reidar Wennesland^{2, 3}

*Physiology Department,
Stanford University, Stanford, California*

A microrespirometer has been developed for studies of metabolism in small animals, tissues, cell suspensions, etc. It is based upon models of volumetric respirometers as constructed by Winterstein (1, 2), Scholander (3, 4), and Wennesland (5). From Scholander (4) has been adopted the use of a plastic block into which a V-shaped manometer has been drilled, connecting the respiration chamber with the compensating vessel.

New features are the inclusion in the plastic block of a chamber for oxygen replacement, and a new measuring device for the gas exchange. The latter has also been developed into a measuring and delivery burette for regular laboratory use (unpublished).

The apparatus is a constant pressure respirometer, maintaining the principal features of Winterstein's original model. The gases are kept under constant temperature and pressure, and the changes in volume are read directly. The theory is thus very simple: no vessel constants need be determined or calculated. The

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³ Present address: The Permanente Hospital, Oakland, Calif.