non-avid substrains derived from the same strain by the procedures described by Choppin and Tamm(9).

It would appear likely that the variations between the strains observed in these experiments, even if they reflect true antigenic dissimilarity, are not of epidemiological significance, since these data suggest that the newer strains react well with antisera to the original 1957 agent.

Summary. Comparison of 1957 and 1960 Asian influenza strains indicated that differences could be detected by both hemagglutination inhibition and complement fixation between some 1957 strains and the 1960 prototype. These differences were detected with antisera against a 1960 prototype virus, but not with antisera against the 1957 prototype employed.

1. Cleeland, R., McKee, A. P., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 425.

2. Takatsy, Cy., Barb, K., *Virology,* 1958, v5, 398.

3. Widelock, D., Klein, S., Peizer, L. R., Simonovic, O., *J.A.M.A.,* 1958, v167, 451.

4. Fukumi, H., *Bull. W. H. O.,* 1958, v20, 421.

5. van der Veen, T., Mulder, T., *Studies on Antigenic Composition of Human Inflenza A Strains with the Aid of Hemagglutination Inhibition Technique,* Leiden, 1950.

6. Lief, F. S., Henle, W., *Bull. W. H. O.,* 1959, v20, 411.

7. Spence, L., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 425.

8. Fulton, F., Dumbell, K. R., *J. Gen. Microbiol.,* 1949, v3, 99.

9. Choppin, P., Tamm, O., *J. Exp. Med.,* 1960, v112, 895.

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Procedure for Bioassaying Mosquito Repellents in Laboratory Animals.* (28486)

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Until recently, insect repellents have mainly been evaluated after surface application on the human arm and visual determination of insect bites by either the experimental subject or the observing investigator(1). This type of evaluation not only suffers from uncertainty of visual determinations but does not take into account the amount of blood ingested by the mosquitoes. Moreover, testing of new compounds with poorly known acute and chronic toxicity, particularly for internal administration, excludes use of the human arm as a testing bait. In our laboratory, the following procedure was found useful in determining the number of mosquitoes biting the mouse used as a bait and the amount of blood taken in by the mosquitoes.

Procedure. The eggs of yellow fever mosquitoes (*Aedes aegypti*) are hatched in deoxygenated water, and the larvae are raised in an incubator (80°F, 80% relative humidity). Pupae are separated according to sex. The adult mosquitoes are housed in the incubator for 4 to 6 days, before testing. Three days before testing, the female mosquitoes are temporarily immobilized by cold exposure (–4°C) and are randomly distributed, 50 in each cage, and fed with 10% sucrose. The following day the sucrose solution is removed. Water is left in the cages until about 4 hours before testing. The prospective repellent is administered to a mouse by an appropriate route at a predetermined time prior to testing. The mouse is injected intraperitoneally 10 minutes before testing with 0.005 ml per gram of body weight of an anesthetic mixture. Each milliliter of the anesthetic mixture contains 80 mg of allobarbital, 320 mg of urethane, and 320 mg of monoethyl urea. Five minutes before testing the indicator solu-

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TABLE I. Effect of Drugs on Mosquito Biting.

Drug	Admin*	Dose	Exp†	Mean wt, mg	Mean blood ingested, μl/50 mosquitoes	Biting, %
None	—	—	16	230‡± 5.2§	283 ± 21§	92 ± 6§
N,N-diethyl toluamide	Local	50% w/v	3	120.2	0	0
Indalone	"	"	1	93.4	0	0
N,N-propylacetanilide	"	"	1	110.5	0	0
Ronnel‖	"	25% w/v	2	—	0	0
Ronnel¶	Intraperitoneal	300 mg/kg	6	—	217	92
Thiamine hydrochloride	Local	1 g/ml	3	—	255	80

* Local application was made 5 min. before exposure, intraperitoneal injection 2 hr before testing.
† Each experiment consisted of 50 mosquitoes exposed to 1 mouse.
‡ Avg wt of starved mosquitoes before exposure was 124.9 ± 1 mg/50 mosquitoes (15 determinations).
§ Standard error.
‖ Trade name (Dow Chem. Co., Midland, Mich.) O,O dimethyl, 0-2,4,5 trichlorophenyl phosphorothioate.
¶ Aqueous suspension with 0.001% Tween 80.

tion containing 20 mg of Blancophore† dye and 20 μc of radioiodinated serum albumin (RISA-Abbott) in each ml, is injected intravenously (0.005 ml/g of mouse weight) into the bait mouse. The bait mouse is placed flat on its abdomen on cheesecloth stretched over the mosquito cage and allowed to remain there for 30 minutes. The mosquitoes are then killed with ether or chloroform.

Immediately after exposure duplicate samples of 25 μl of blood are drawn from the orbital cavity of the mouse by the bleeding technique of Riley(2). The blood of the mouse and of the mosquitoes exposed to the same mouse is then radioassayed by standard scintillation counting method. The amount of blood ingested by the mosquitoes is then calculated from the level of radioactivity in the mouse blood and that in the exposed mosquitoes. The mosquitoes are also weighed and counted under ultraviolet light to determine the number which have bitten the mouse. The mosquitoes which feed on the mouse appear bright green, while those that do not feed remain black.

In separate experiments it was determined that the dye (Blancophore) or the radioactive albumin did not repel mosquitoes to any noticeable extent. Also, the level of radioactivity in the mosquitoes was directly proportional to that in the blood or the injected dose. To illustrate the reliability of the technique, results from repellents of known interest are included in Table I. Results from other potential mosquito repellents will be published later.

From these data it is clear that local application of popular mosquito repellents prevented mosquito biting completely during the test period, while local application of concentrated thiamine solution or intraperitoneal administration of Ronnel did not have any repellent effect. The compounds producing partial repellency will be reported later.

Discussion. Radioactive elements for measuring blood-meal size in mosquitoes have been used before(3,4). Employment of P^{32}-labeled phosphoric acid is not suitable for *in vivo* tests because of the rapidity with which it is removed from the blood circulation by tissue incorporation and excretion. Furthermore, the radioactivity contained in the mosquito excreta may contaminate non-radioactive mosquitoes. Use of Ce^{144}(5) is limited by its high cost, its bone-seeking properties, and several health hazards associated with the use of this isotope. In our laboratory, the experiments conducted with urea-C^{14} did not show reproducible results. Presumably this is due to the large variation in tissue concentrations from animal to animal(5). Secondly, urease of the mosquitoes may hydrolyze urea after ingestion. Also, considerable dilution of urea occurs in the animal due to large urea spaces in the tissues(5). This requires that a high activity be injected to detect the radioactivity in mosquitoes. On the other hand, radioiodinated serum albumin is mixed uniformly in a very short period and remains restricted to

† Blancophore SVT-400, General Dyestuff Co., New York.

vascular compartments. During the period of our experiment radioactivity in the blood remained constant and uniform, as was evident from radioassay of blood samples withdrawn at 5 minute intervals and similar specific radioactivity found in blood drawn from the right or left eye.

The bioassay proved useful and reliable in our laboratory under a variety of testing conditions. While our experience has been exclusively with mice, the technique can be used with other small laboratory animals. It eliminates the need for such methods of detecting mosquito biting as looking for mosquitoes with distended abdomens, looking for the sign of a bite on shaved skin, or visual inspection of landing, procedures of obviously doubtful value.

Summary. A laboratory method for measuring the number of mosquitoes that had bitten and the amount of blood ingested during exposure is described. The procedure was successfully used in testing mosquito repellents administered to living mice.

1. Dethier, V. S., *Ann. Rev. Entomol.*, 1956, v6, 181.

2. Riley, V., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 751.

3. Bar-Zeev, M., Schmidt, C. H., *J. Econ. Entomol.*, 1959, v52, 268.

4. Boormal, J. P. T., *Ann. Trop. Med. and Parasit.*, 1960, v54, 8.

5. Lal, H., Ph.D. Thesis, Univ. of Chicago, 1962.

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Separation and Quantitative Recovery of Iron and Cobalt from Biological Samples.* (28487)

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Trace metal analysis in biological samples has been accomplished with difficulty by conventional means. Recent reports of activation analysis demonstrate its extreme sensitivity and usefulness in this area(1). Neutron activation of biological samples produces an array of isotopes whose gamma ray spectra may obscure the element for which quantitation is desired. When the specimen contains large numbers of elements at extremes of concentration the various radio-spectral techniques for quantitation become either impractical or imprecise. The quantitation of Fe^{59} and Co^{60} in samples exemplifies these problems(2). The method outlined below is a relatively simple ion-exchange column separation for iron and cobalt. While developed specifically for separation of these 2 elements in activated specimens, its demonstrated excellent quantitative recovery would suggest broader applications for biological trace metal research.

Materials and methods. Pyrex columns 26 cm × 1 cm topped by an 8 cm funnel were used. These were plugged with glass wool and 15 ml of a slurry, made one part Dowex-1 (100 – 200 mesh) to one part distilled water, were added. The columns, which were filled to aproximately 18 cm were then charged utilizing concentrated HCl.

High specific activity ferrous citrate§ (Fe^{59}, 26.5 mc/mg) and cyanocobalamin§ (Co^{57}, 750 μc/mg B_{12}) were added to fresh pooled bovine serum. The final concentration of added ferrous citrate was approximately 0.02 μg/ml plasma and of B_{12} approximately 0.5 μg/ml plasma. One ml aliquots of this plasma were then pipetted into small aluminum foil containers which were heated in a drying oven at 100°C until the plasma became

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