From these measurements it appears that the incidence of eye lesions is directly related to length of exposure to the low intensity colored light, with an increase in the incidence of eye lesions occurring in those remaining in the red light.

Summary. Birds reared in low intensity colored light developed greatly enlarged eyes. Associated with the eye enlargement was: (1) a change in dioptrics, (2) increased eye protrusion, (3) increased incidence of eye lesions, especially in the red spectrum, which was directly related to length of exposure to the low intensity light, and (4) a thickening of the eye wall which occurred mainly in the choroid layer. The appearance was characterized by exophthalmos rather than the buphthalmos which occurs in continuous light. Corneal curvature was not changed while other eye parameters increased

1. Gorn, R. A., Kawabara, I., Arch. Opthalmol., 1967, v77, 115.

2. Jensen, L. S., Matson, W. E., Science, 1955, v125, 741.

3. Lauber, J. K,. McGinnis, J., Vision Res., 1966, v6, 619.

4. Lauber, J. K., Shutze, J. V., McGinnis, J., Proc. Soc. Exp. Biol. & Med., 1961, v106, 871.

5. Noell, W. K., Themann, H., Kang, B. S., Walker, V. S., Fed. Proc., 1966, v25, 329.

6. Noell, W. K., Walker, V. S., Kang, B. S., Bermann, S., Invest. Ophthal., 1966, v5, 450.

Received June 23, 1967. P.S.E.B.M., 1967, v126.

Substitution of Egg Yolk for Serum in Indirect Fluorescence Assay For Rous Sarcoma Virus Antibody. (32433)

CALVIN G. AULISIO AND ALEXIS SHELOKOV

Department of HEW, U. S. Public Health Service, National Institutes of Health, Division of Biologics Standards, Laboratory of Virology & Rickettsiology, Bethesda, Md.

While searching for simpler and more rapid methods of monitoring chicken flocks for evidence of avian leukosis infection, we noted that other investigators had substituted egg yolk for mother hen's serum in neutralization and metabolic inhibition tests with Rous sarcoma virus(1,2). By using egg yolk as antibody reagent in an indirect immunofluorescence system we have developed a simple and rapid test for detection of viral antibody in birds.

Material and methods. Viral antigen. Three strains of Rous sarcoma virus (RSV) were used: Schmidt-Ruppin—lot SR45, Harris—lot HA8, and CT559—lot TV43, all of which were kindly supplied by Dr. R. Holdenried, National Cancer Institute, Bethesda, Md. A strain of avian lymphomatosis virus (RIF), RPL 12-L37, was provided by Dr. W. Okasaki, Regional Poultry Laboratory, East Lansing, Mich.

Chick embryo fibroblasts (CEF), grown in 32 oz bottles as primary culture, were suspended in a medium composed of the following ingredients (parts per 100): Eagle's #2 medium—88; tryptose phosphate broth —5; calf serum—5; stock 3% glutamine solution—1; stock antibiotic solution—1 (to give a final concentration of penicillin 200 u per ml and streptomycin 200 mcg per ml). To infect the cultures with RSV, 0.1 ml of a virus dilution, containing approximately 2-3 logs of virus, as determined by the fluorescent antibody (FA) technique, was added to 10 ml of the cell suspension containing 3×10^6 CEF. The RSV-infected cells were seeded on pre-cleaned coverslips in a Falcon plastic Petri dish (100 × 20 mm). To infect the cultures with RIF virus, 1 ml of the virus dilution containing approximately 10,000 $TCID_{50}$ as determined by RIF test(3) was added to 50 ml of the cell suspension (15×10^6 CEF) seeded in 32 oz bottles. The RIF virus-infected cells were passed twice after 3 or 4 days' growth in bottles; the third passage cells were seeded in a Falcon plastic Petri dish, as described for the RSV procedure. The Petri dish cell cultures were incubated in a

humidified CO_2 (5%) incubator. After 3 or 4 days, when the cells had formed a confluent sheet, the coverslips were removed and washed in pH 7.2 phosphate-buffered saline (PBS) for several seconds, fixed in cold acetone (—20°C) for 10 minutes, air-dried and stored at —60°C in rubber-stoppered glass shell vials.

Antibody. Eggs obtained from hens with and without predetermined serum levels of Rous sarcoma virus antibody (RSVA) were selected. The method of extraction of yolk antibody was similar to that used by Rubin *et al*(1) and Kottaridis *et al*(2). Equal volumes of yolk (without albumin) and saline were placed in a screw-cap tube and mixed by shaking; a double volume of chloroform was added next and the tube was inverted several times after which it was left at room temperature for ½ hour. The procedure was repeated at least 4 more times at ½ hour intervals and the mixture was placed in the refrigerator (5°C). The next morning it was spun in an International PR-2 centrifuge (head No. 253) for 10 minutes at 2,000 rpm and the upper, clear saline layer (Fig. 1) was pipetted off. The middle layer of depigmented yolk debris and the lowermost, pigmented chloroform layer were discarded.

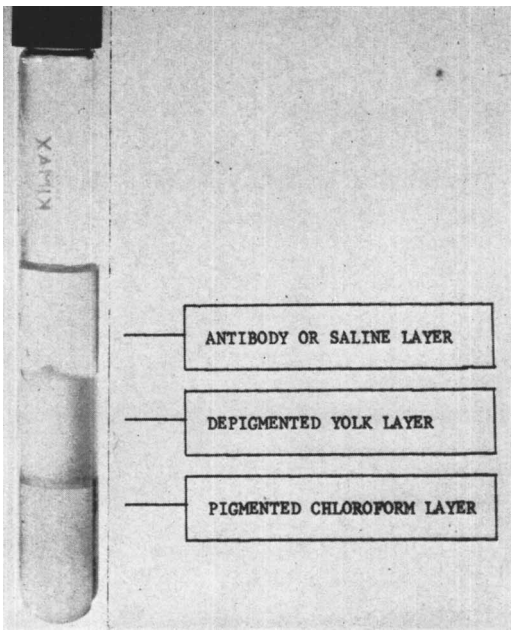

FIG. 1. Chloroform extract of RSV antibody in egg yolk after centifugation.

The saline extract, designated a 1:2 dilution of the yolk, was used immediately or was stored at —60°C. Preliminary testing showed that 4-fold dilution of the extract, *i.e.*, 1:8 dilution of the yolk, provided an adequate working antibody reagent without troublesome nonspecific fluorescence. The hens' sera could not be satisfactorily used at a less than 1:40 dilution and even then some nonspecific fluorescence usually persisted.

Indirect FA technique. Virus-infected and uninoculated control coverslips were flooded with a hen's serum or yolk extract and incubated at room temperature for 30 minutes. The coverslips were rinsed with PBS and left soaking for 10 minutes to help remove the unreactive antibody reagent; they were then stained for 30 minutes with a mixture of fluorescein-labeled antiserum, prepared against chicken globulin, and rhodamine-conjugated bovine serum albumin. The PBS washing was repeated and the coverslips were mounted in buffered glycerol (pH 7.5) for examination with a binocular AO fluorescence microscope. The filter system consisted of a Schott BG12 or a Corning 5840 exciter filter and a Schott OG1 or GG9 barrier filter.

Results and discussion. Table I presents the results of an immunofluorescence survey of 5 chicken flocks for the presence of RSVA. Two flocks (flocks A and B) were housed under conditions which were not expected to prevent the spread of RIF virus infection; the other 3 flocks (flocks C, D and E) were said to be reared under RIF-free conditions. Examination of 41 sera from hens in one of

TABLE I. Immunofluorescence Survey of Five Chicken Flocks for RSV Antibody.

Flock	Breeders' designation	Material	No. tested	No. positive for RSVA
A	Not RIF-free	Serum*	41	18
		Yolk†	10	4
B	" "	Yolk	12	9
C	RIF-free	"	22	0
D	"	"	20	0
E	"	Serum	220	0
		Yolk	95	0

* Serum—lowest dilution tested 1:40.
† Yolk—lowest dilution tested 1:8.

the flocks potentially contaminated with RIF virus (flock A) showed that 18 birds (44%) had RSVA at the 1:40 (minimal) serum dilution. Four of 10 eggs obtained from the same flock, but not necessarily the same birds, had RSVA in their yolks when tested at the 1:8 dilution. Nine of 12 eggs from another similar facility (flock B) were also positive for RSVA. Small numbers of eggs (22 and 20), but not sera, were available from two RIF-free establishments (flocks C and D) and these were negative for RSVA; sera (220) and unmatched eggs (95) were obtained from hens in the third establishment (flock E) and all were RSVA negative.

To compare the antibody levels of the serum and egg yolk from the same birds an experiment was designed using flock A. The results are summarized in Table II. Six of 18 birds with RSVA (hens No. 1-6) and 2 of 23 birds without this serum antibody, as judged by the results of tests done at the 1:40 dilution of the serum (hens No. 7 and 8), were trap-nested and bled on the day of egg collection. The yolks and the sera were tested (beginning with the usual working dilutions of 1:8 and 1:40 respectively) against Schmidt-Ruppin, Harris, CT559 and RIF viruses. The yolks from eggs of hens with known serum antibody reacted with each of the 4 antigens. The yolk antibody level was never higher than the serum level; usually the yolk level was the same or somewhat lower. This is consistent with the earlier reports employing neutralization(1) and metabolic inhibition(2) tests to compare the RSVA content of hen's serum and egg yolk. The egg yolk from one of the control hens reacted at the 1:8 dilution with 2 of 4 antigens. This suggests that the RSVA in the hen's serum may have been missed during the initial screening at the usual 1:40 dilution.

Treatment with either hen's serum or yolk extract produced characteristic fluorescent cytoplasmic granules in RSV-infected cells (Fig. 2a & b), but not in the uninoculated control cells. Specific fluorescence was not

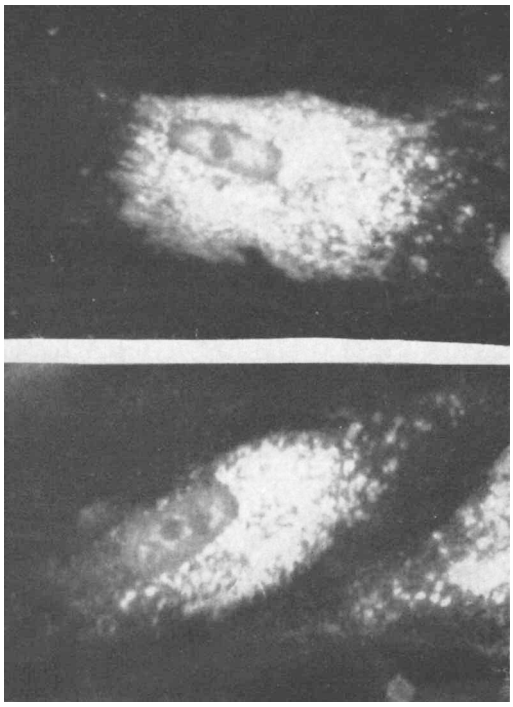

FIG. 2. Indirect immunofluorescence of RSV, Harris strain, with hen's serum (upper) or yolk extract (lower) as intermediate antibody reagent.

TABLE II. Comparative Immunofluorescence of 8 Selected Hens' Sera and Matching Egg Yolks with 4 Avian Virus Antigens

Hen No.	1		2		3		4		5		6		7		8	
Antigens	S*	Y†	S	Y	S	Y	S	Y	S	Y	S	Y	S	Y	S	Y
Schmidt-Ruppin	1280‡	640	640	640	640	160	320	160	320	160	160	160	0	8	0	0
Harris	640	320	320	160	160	160	160	160	160	160	160	160	0	0	0	0
CT 559	320	80	NT§	NT	NT	NT	NT	NT	NT	NT	NT	NT	0	8	NT	NT
RIF	640	80	160	80	160	80	160	80	160	40	160	40	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Serum—lowest dilution tested 1:40.
† Yolk—lowest dilution tested 1:8.
‡ Results expressed as reciprocal of serum or yolk dilution endpoint.
§ NT—not tested.

seen in RSV- and RIF-infected cells treated with either sera from RIF-free birds or yolk extracts from their eggs.

Compared to the other procedures utilizing egg yolk rather than mother hen's serum to assay for RSVA(1,2), our method offers the advantage of rapidity (hours rather than days) and simplicity in that it does not require the exacting adjustments of cell culture population or medium composition.

Conclusions. Substitution of egg yolk for the mother hen's serum does away with the cumbersome procedure of bleeding the birds for antibody tests. The level of RSVA in yolk reflects the serum antibody level but is usually somewhat lower. Furthermore, substitution of egg yolk for the mother hen's serum in an indirect immunofluorescence procedure provides a simple, rapid, reliable and relatively inexpensive testing system. Flocks of birds can be easily examined and in most cases the test results are available the same day.

The authors are grateful to Dr. Nicola M. Tauraso and Mr. William G. Jahnes of this laboratory who generously contributed of their time and materials to make this study possible.

1. Rubin, H., Fanshier, L., Cornelius, A., Hughes, W. F., Virology, 1962, v17, 143.

2. Kottaridis, S. D., Chomiak, T. W., Luginbuhl, R. E., Avian Dis., 1967, v11, 65.

3. Rubin, H., Proc. Nat. Acad. Sci., 1960, v46, 1105.

Received June 26, 1967. P.S.E.B.M., 1967, v126.

Insulin Half-Life in Normal and Diabetic Subjects.* (43434)

T. TOMASI, D. SLEDZ, J. K. WALES, AND L. RECANT

Department of Medicine, Georgetown University School of Medicine, Washington, D.C., Veterans Administration Hospital and Research Foundation of the Washington Hospital Center

Recent reports have suggested the likelihood that I^{131} labelled insulin preparations may have altered biological properties when compared with unlabelled insulin(1,2). As a result, previous studies of the turnover of insulin in normal and diabetic subjects conducted with labelled insulin have become suspect. Samols *et al*(3) in 1 normal patient and Orskov *et al*(2) in forearm studies in 5 normal patients have studied respectively the disappearance rates of plasma insulin following infusion of glucagon or exogenous unlabelled insulin, in an effort to obtain the true t½ of insulin. Both groups used immunoassay procedures for insulin measurements. This is in sharp contrast to the measurements of plasma TCA precipitable radioactivity or to the chromatographic distribution of radioactivity used in the *in vivo* I^{131} insulin experiments. The t½ arrived at by immunoassay was of the order of 5-15 minutes with mean values of approximately 7.5 minutes, a considerably shorter time than reported with radioactive insulin studies.

* Supported in part by USPHS Grant 5 RO1-HE 10102.

The t½ of insulin in untreated diabetics, based on radio-insulin studies, has been reported to be the same as in normals(4). In view of the substantially shorter t½ observed with the methods of Samols and also of Orskov in normals, it seemed important to restudy untreated diabetics. The present investigation reports studies in 6 normal subjects and 5 newly diagnosed untreated diabetics. No significant differences in insulin disappearance rates were observed following the administration of 2 units of *unlabelled* crystalline bovine insulin I.V.

Methods and materials. Normal young medical students served as control. All were in good health, on good diets, and with no evidence of carbohydrate abnormalities. The diabetic subjects were in-patients at the Washington, D. C., Veterans Administration Hospital. None had ever received insulin and were on diets containing at least 200 g of carbohydrate. All subjects were fasted overnight. After the fasting samples were obtained and 2 units of crystalline bovine insulin (E. Lilly Co.) were injected rapidly, an indwelling needle was placed in the opposite